BRIAN R. JOHNSON

WITH A FOREWORD BY THOMAS D. SEELEY

HONEY BEE BIOLOGY

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CONTENTS

List of Plates vii
Foreword ix
Acknowledgments xiii

1 Introduction 1
2 Natural History, Systematics, and Phylogenetics 4
3 Development 23
4 Anatomy and Physiology 47
5 Genetics and Genomics 76
6 Neurobiology 90
7 Neuroethology and Cognitive Science 116
8 Reproduction 137
9 Evolution 159
10 Life History, Ecology, and Nesting Biology 182
11 The Honey Bee Colony Is a Superorganism 196
12 Division of Labor 204
13 Communication, Labor Allocation, and Collective Decision Making 226
14 Chemical Ecology 250

15 Foraging 272

16 Tropical Honey Bees 290

17 Immunity, Parasites, Pests, and Pathogens 301

18 Detoxification and Pesticides 322

19 Honey Bees as Managed Pollinators 338

Literature Cited 353
Index 477

PLATES

1. Life cycle stages of the worker honey bee from egg to adult. Photo courtesy of Kathy Keatley Garvey.
2. Honey bee egg. Photo courtesy of Kathy Keatley Garvey.
3. Queen cups. Photo courtesy of Kathy Keatley Garvey.
4. Queen bee. Photo courtesy of Kathy Keatley Garvey.
5. Worker with drone. Photo courtesy of Kathy Keatley Garvey.
6. All three honey bee castes inside the nest. Photo courtesy of Kathy Keatley Garvey.
7. Stinging honey bee. Photo courtesy of Kathy Keatley Garvey.
8. A small swarm in a tree. Photo courtesy of Kathy Keatley Garvey.
9. Bee secreting wax flakes. Photo courtesy of Kathy Keatley Garvey.
10. Newly emerging bee. Photo courtesy of Kathy Keatley Garvey.
11. Bee foraging on lavender. Photo courtesy of Kathy Keatley Garvey.
12. Bees collecting water. Photo courtesy of Kathy Keatley Garvey.
13. An observation hive. Photo courtesy of Kathy Keatley Garvey.
14. Laying queen with retinue. Photo courtesy of Kathy Keatley Garvey.
15. Varroa mites on developing bee. Photo courtesy of Kathy Keatley Garvey.
16. Colonies being used for almond pollination. Photo courtesy of Kathy Keatley Garvey.

FOREWORD

Thomas D. Seeley

In history and in science, the honey bee (*Apis mellifera*) has always been our foremost social insect. One hundred years ago, in 1923, the Harvard entomologist William Morton Wheeler observed that in antiquity this bee's intimacy with flowers, its avoidance of all things unwholesome, its astonishing industry in storing honey, and its skill in making wax made the honey bee "a divine being, a prime favorite of the gods, that had somehow survived the golden age or had voluntarily escaped from the garden of Eden with poor fallen man for the purpose of sweetening his bitter lot." Now, a century later, the honey bee has become one of the most intensively studied animal species, especially with reference to its behavior and social life.

Research on honey bee biology over the past century can be divided into four principal periods. The first was led by Karl von Frisch from the mid-1910s to the 1950s. In these years, the focus was on the sensory abilities and behavioral skills of individual honey bees. Much was learned about the visual, olfactory, and time-sense abilities of worker bees. Other prime targets of investigation were their division of labor by age, communication using the famous waggle dance, and orientation skills outside the hive by reference to landmarks and skylight cues.

The second period began in the early 1950s. This is when studies of honey bee communication by pheromones took off, as behavioral biologists developed clever bioassays and organic chemists acquired powerful analytic tools, such as gas chromatography. The 1950s was also the time period when the most gifted student of Karl von Frisch, Martin Lindauer, began his pioneering studies of honey bee social behavior, including their division of labor (by age) and their collective decision making when choosing a home site. These are also the years when Lindauer conducted his path-breaking work on the other species in the genus *Apis*, all of which live in southern Asia. This work helped develop the perspective that the distinctive traits of *Apis mellifera*—colony fissioning (swarming) for reproduction, and colony survival over cold winters as self-heated clusters inside snug nest cavities—are adaptations of a social insect that arose in the Asian tropics and that expanded its range westward and northward, into the temperate zones.

The third period of research, which augmented but in no way supplanted the work in the first two, gained impetus in the 1970s when biologists studying honey bees came under the influence of the new disciplines of sociobiology and behavioral ecology. At this stage, investigators began to address questions inspired by natural selection theory. One example, from the field of sociobiology, is this: Why do workers rarely use their ovaries to produce unfertilized eggs (and have sons)? Is the "queen substance" pheromone that a queen produces in her oversized mandibular glands a drug that inhibits worker reproduction, or is it a signal that informs the workers of her presence? It turned out that "queen substance" is not a drug whereby the queen controls the workers' reproduction. Instead, what inhibits their egg laying is a kind of "policing" by fellow workers: any worker-laid eggs are quickly eaten by other workers. Another example from this third period comes from the domain of behavioral ecology: How do workers use their famous waggle dance? By recording and translating the waggle dances performed by successful foragers, to find out the directions and distances of the flower patches they were visiting, and then plotting the patches' locations onto circular maps with the hive at the center, biologists were able to track the shifting foci of a colony's foragers as if they were objects on a radar screen. This yielded several surprises. One was that a colony "patrols" an area greater than 100 square kilometers for food, and that the honey bee's exquisite communication system enables a colony's foragers to shift the focus of their activity across the immense terrain as the foraging opportunities change. This work helped bolster the view that a honey bee colony is a superorganism, that is, a group-level "vehicle" whereby the genes of its members are passed fairly into the future.

The fourth period, which is the present, builds on the work of the previous three. Now the powerful tools of neurobiology, quantitative and evolutionary genetics, and molecular biology are being used to examine more deeply than ever before the mechanisms that underlie the countless striking contrasts found within the biology of honey bees. These include the contrasts in physiology and behavior between queens and workers, between workers functioning as nurses and foragers, between foragers that do and do not collect pollen, and between high- and low-elevation races of honey bees in Africa (i.e., *Apis* *mellifera monticola* and *A. m. scutellata*). In short, these studies are revealing much that, until the 1990s, was a “black box” of mechanisms of physiology and behavior.

Because honey bees have attracted intensive study over a broad range of topics, investigators of these bees were helped greatly in the past by two book-length reviews of the scientific literature on their biology: Ronald C. Ribbands’s book *The Behaviour and Social Life of Honey Bees* (1953) and Mark L. Winston’s book *The Biology of the Honey Bee* (1987). Now, Brian R. Johnson’s book, *Honey Bee Biology*, takes its place among these important works of synthesis. It is a wonderful, and indeed an amazing, exemplar of this approach.

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No person is an expert on all of biology and this book would not have been possible without advice on early drafts from many bee scientists from around the world. In alphabetical order, I would like to thank Kirk Anderson, Martin Beye, Vanessa Corby Harris, Bryan Danforth, Christopher Dearden, Jamie Ellis, Jay Evans, Julia Fine, Daniel Friedman, Cole Gilbert, Klaus Hartfelder, Martin Hassellmann, Timothy Linksvayer, Stephen Martin, Randolph Menzel, James Nieh, Robert Page, Gene Robinson, Olav Rueppell, Jean-Christophe Sandoz, Stan Schneider, Marla Spivak, Srini Srinivasan, Paul Szyszka, David Tarpy, and Amro Zayed. Finally, I would like to thank Thomas Seeley, who has been a wonderful mentor to me from graduate school to the present. He gave invaluable advice on several chapters.

Honey Bee Biology

1

Introduction

Honey bee biology does not need much selling to attract the nontechnical reader, or the applied scientist working in agriculture. But are honey bees as interesting and important for basic scientists? The answer is yes. The honey bee is in fact one of the best-understood organisms from an integrative biology perspective. A search of any scientific search engine, for example, will locate thousands of papers about honey bee biology. The majority of these are not about agriculture, or any aspect of applied bee biology, but rather focus on the basic science of bees. Studies of their systems of communication, the developmental mechanisms leading to queen versus worker morphology, and division of labor, for example, have vast bodies of work.

This fascination with bees might need some explaining. Of course, model systems in biology, like the fruit fly, are the subject of many more studies than are honey bees. However, the fruit fly is a model for genetics, and the overwhelming majority of fruit fly studies are about that subject. There is considerable work on other aspects of fruit fly biology, but in general many aspects of their biology are understudied. In a sense, this is because these animals serve as medical models that we use to address biological questions of practical concern. This is generally the case for model systems.

In contrast to the model systems, the honey bee, until recently, was studied by biologists mainly because it is interesting and because we like bees. In other words, science simply for the sake of knowledge drives quite a lot of honey bee biology. Because of this, we know a great deal about every aspect of bee biology, both at the molecular and the organismal levels. This is not to say that the honey bee is not a model, as well, for some questions. The honey bee is in fact something of a model system for social insect biology. Social insects are the most complex animal societies, and they are ecologically dominant in many habitats. Among the social insects, the honey bees are not the most complex, but they are the most amenable to study. The long history of beekeeping, which provides many tools for the scientist, ensures that they are easier to work with than insects with no history of management. Hence, researchers interested in social behavior, pollination, communication, and many other topics naturally gravitate to the honey bee as a subject organism.

Having covered in broad strokes why the honey bee attracts so much attention, we now turn to the other major question of the introduction. Why this book and why now? The answer is simple. There is a wonderful reference for the basic biology of the honey bee, Mark Winston's *The Biology of the Honey Bee*. This has long been on the shelves of scientists interested in bees. Beekeepers interested in acquiring a deeper understanding of the creatures they love have also made much use of this work. However, Winston's book is now over 30 years old and is out of date on many subjects. It is chiefly lacking in two ways. First, many of the subjects covered in the Winston book have changed radically in scope, with major new approaches having uncovered phenomena unknown when that book was published. Second, there are now several fields in biology that, although present 30 years ago, were little studied, and hence did not get covered in Winston's book. Some of these fields are now larger than some traditional fields; examples include toxicology, pollination, and immunity. Hence, the goal of this book is to provide a new standard reference for honey bee biology that explores this fascinating insect from both traditional and new scientific perspectives.

To the Beekeeper

This is a book for scientists about the biology of honey bees, so one might be surprised to find a section addressed to beekeepers. The surprised person would not be too familiar with beekeepers, however, since this group of enthusiasts is so fascinated by the colonies they care for that they routinely buy books such as this and invite practicing scientists to talk at their beekeeping clubs. I personally have seen the Winston book in the hands of many beekeepers. Hence, I want to provide a brief guide to reading this book for the nonscientist.

In general, any topic that does not take a molecular approach should be approachable for a beekeeper. This includes most of the work on anatomy and physiology, taxonomy, reproduction, neuroethology, division of labor, task allocation, chemical communication, nesting biology, parasites and pathogens, tropical bees, and pollination. These are the topics typically of most interest to beekeepers. Although there is some molecular biology in these sections, it is not central to understanding the science. The chapters on development, evolution, genetics, and neurobiology, in contrast, are probably too technical for the lay reader. However, I think with some determination the beekeeper could grasp the key ideas even in these chapters. I say this because there are now so many free sources of information to get a better understanding of background material. I imagine with some background reading, and maybe viewing of some science tutorials on YouTube, that quite of lot of the technical material might become transparent.

2

Natural History, Systematics, and Phylogenetics

The Bees

Bee and *honey bee* are often used interchangeably in public discussions. Nothing frustrates the systematist more, as there are over 20,000 described species of bees in the world (Michener, 2000). The bees evolved from the hunting wasps, a group of four clades of wasps that typically provision their offspring with insects or spiders. Probably the most well known of the hunting wasps (to the nonentomologist) are the mud daubers that build their nests on the sides of people's homes. The split between these wasps and what evolved into the bees occurred about 120 million years ago (Cardinal and Danforth, 2013). Bees nest within this clade, which in lay terms means that bees are evolutionarily closer to some hunting wasps than those hunting wasps are to other hunting wasps. As Danforth et al. (2019) remarked, bees are thus basically hunting wasps that have gone vegetarian.

Bees fall into seven families (28 subfamilies), the most recent phylogeny of which is shown in figure 2.1 (Danforth et al., 2006, Lo et al., 2010, Bossert et al., 2019, Danforth et al., 2019). The Melittidae are a small group of ground nesting bees that tend to be plant specialists. The Megachilidae, Colletidae, and Andrenidae are common cosmopolitan families of solitary bees that contain many important pollinators. The Stenotritidae are the smallest family and are limited to Australia. The Halictidae, commonly called sweat bees, are common across much of the world and are widely studied from both basic and applied perspectives (Brady et al., 2006, Kocher et al., 2013). They tend to be bright metallic colors and get their common name from the practice of occasionally collecting salt from human sweat. Most of the bees with common

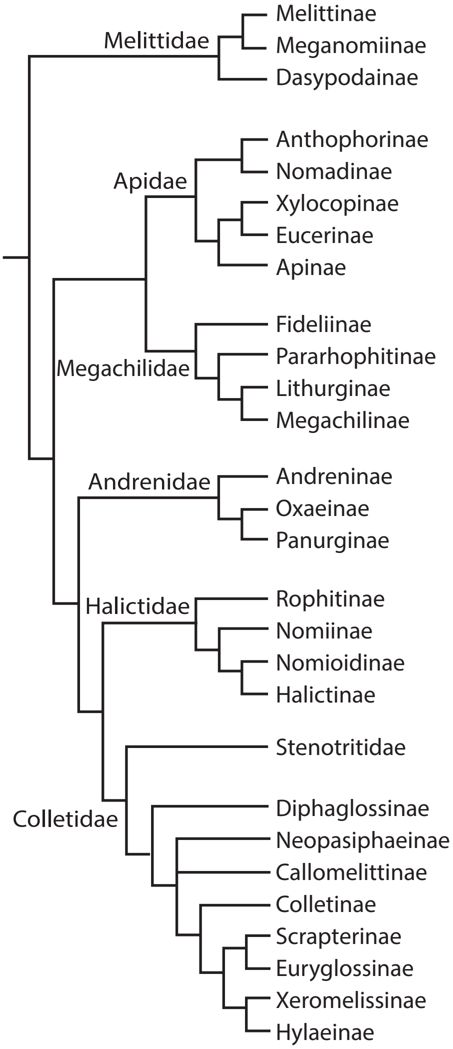
Melittidae
Melittinae
Meganomiinae
Dasypodainae
Apidae
Anthophorinae
Nomadinae
Xylocopinae
Eucerinae
Apinae
Megachilidae
Fideliinae
Pararhophitinae
Lithurginae
Megachilinae
Andrenidae
Andreninae
Oxaeinae
Panurginae
Halictidae
Rophitinae
Nomiinae
Nomioidinae
Halictinae
Stenotritidae
Colletidae
Diphaglossinae
Neopasiphaeinae
Callomelittinae
Colletinae
Scrapterinae
Euryglossinae
Xeromelissinae
Hylaeinae

FIGURE 2.1. Phylogenetic tree of the bees (*redrawn from Danforth et al., 2019*).

names are in the family Apidae, which includes honey bees, bumble bees, stingless bees, and orchid bees.

Unlike some taxonomic groups, the classification of bee families does not correspond to obvious life history differences. Bees across families have similar morphology (to the nonspecialist) and behavior, and in many cases a trained taxonomist is required to place a given bee into the right family. For this reason, functional categorizations of bees have become popular with ecologists and behaviorists. Social or solitary, pollination specialist or generalist, or short tongued are examples, as these groups are not based on phylogenetic history (Michener, 2000).

Basic Bee Life History

Bees have four basic life history strategies: solitary, social, brood parasites, and social parasites (Danforth et al., 2019). We start our discussion with the parasites. Brood parasites do not build or provision their own nest. Somewhat like cuckoo birds, they invade another nest and lay their eggs on the provisions collected by another bee (usually a closely related species). Either the parasitic larva or the mother bee kills the offspring of the host. Similarly, social parasites do not found their own nests, but rather take over established colonies (again, usually of closely related species). The colony then rears the offspring of the parasite, who takes over as the egg layer. Social parasites are common in the bumble bees, and as we see later in the book, it has been suggested that *Apis mellifera capensis,* a subspecies of African honey bees, either is evolving into a social parasite or already is one, engaging in this behavior in a facultative fashion (Neumann and Moritz, 2002).

The overwhelming majority of bee species in all families are solitary (Michener, 1974, Wcislo and Danforth, 1997, Danforth et al., 2019). Solitary bees have a life history illustrated in idealized form in figure 2.2, which focuses on the female (a practice common in the study of solitary and social bees). After mating, the female builds a nest in which she provisions one egg after another with pollen. Each egg gets its own chamber. Most nests are thus tubular. Females die at the end of the season, and the next generation overwinters, typically as prepupae or adults, although there is much variation across groups with some overwintering as larvae (Danforth et al., 2019). The following spring, the next generation emerges, mates, and repeats the process. This basic pattern, quite variable in its details, can serve as a backdrop for thinking about how much evolution has changed the lifestyle of social bees. Perhaps the major ecological difference between solitary and social bees is that most solitary bees are specialists and have a phenology closely linked to the plants they use, while social bees are nearly all plant generalists and are active from spring to fall.

Nesting biology, across the bees, has more variability than is commonly known. Danforth et al. (2019) recently argued for four categories: ground

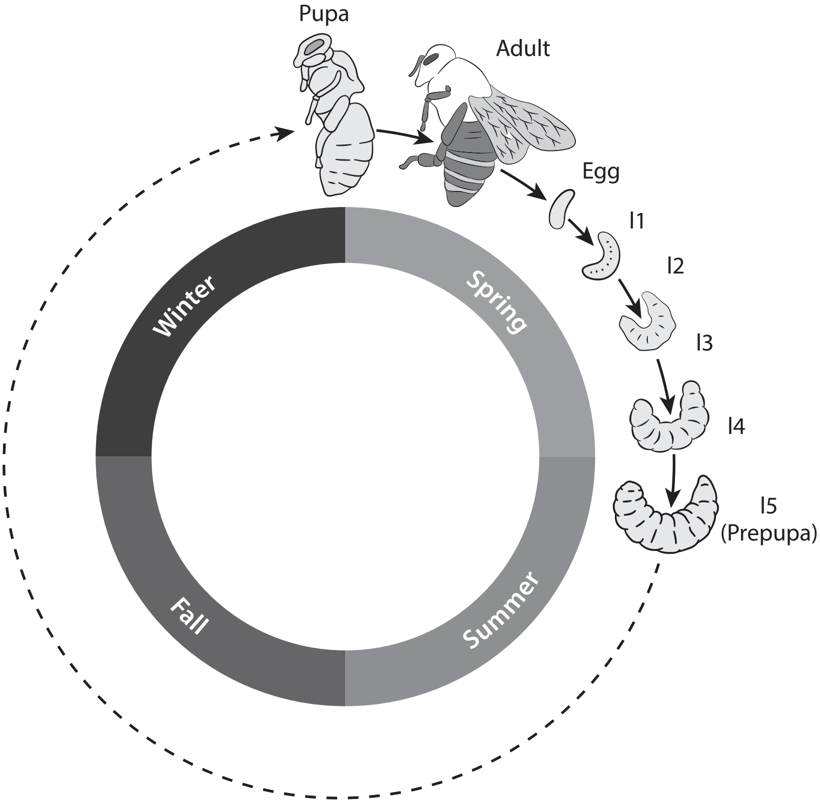

FIGURE 2.2. Idealized life history of a solitary bee (*redrawn from Danforth et al., 2019*).

excavators, wood excavators, renters, and aboveground builders. Bees that excavate a nest in the soil are the most common. Wood excavators include the carpenter bees and the many groups that excavate pithy stems. Renters use preexisting cavities of many sorts, including old nests from many other insect species and a vast array of other tubes of biotic or abiotic origin. Aboveground builders construct a nest from various plant materials or mud.

Apidae Natural History

The family Apidae is composed of about 6000 species divided into five subfamilies. Apidae is the largest family of bees, and the oldest known fossils come from this group (Danforth et al., 2019). The honey bee is in the subfamily

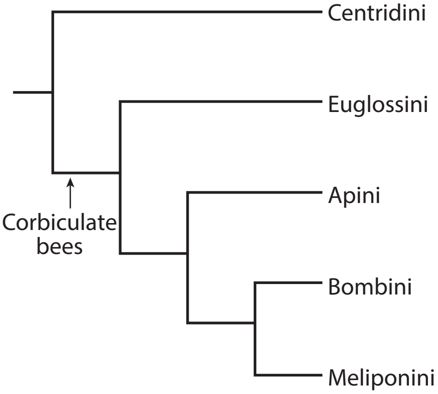

FIGURE 2.3. Evolutionary relationships between the corbiculate bees *(redrawn from Danforth et al., 2019)*.

Apinae (1200 species), which has five tribes: Centridini (all solitary, no common name), orchid bees (Euglossini), bumble bees (Bombini), stingless bees (Meliponini), and honey bees (Apini). The last four groups make up the corbiculate bees, all of which have a pollen basket on the hind legs (described in chapter 4). The most recent phylogeny is shown in figure 2.3. This clade is thought to have four to five origins of eusociality (reviewed in Danforth et al., 2019). Orchid bees are the least social, as most are solitary or communal (nest in aggregations) with only a minority of social species. The bumble bees are all either eusocial (with small colonies) or social parasites. Honey bees and stingless bees are highly derived eusocial. The stingless bees have great variability in their social structure (colony size, presence of physical castes, life history, etc.) and are quite speciose. The honey bees are the opposite, with little life history variation and few species. Honey bees stand out, however, as being ecologically dominant (in their native and nonnative ranges).

In general, the honey bee can be said to reside in the family that is the hot spot for social evolution in the bees, as everything from solitary to the highest forms of sociality are present. This cannot be said for any other bee clade, for although halictids also show much interesting social evolution (lots of reversions to solitary living, for example), there are no large colony perennial halictid societies.

Natural History of Eusociality

About 9.4% of bees are social and these are spread across two families: the Halictidae and the Apidae (Michener, 2000, Danforth, 2002, Danforth et al., 2019). Following the traditional convention, eusocial behavior is broken up into the incipiently eusocial, the primitively eusocial, and the advanced eusocial (Michener, 1969, Johnson and Linksvayer, 2010). Eusociality refers to the presence of three traits: overlapping generations (mothers and daughters in the same nest), cooperative care of brood, and a reproductive division of labor (Wilson, 1971). In place of primitive and advanced, we prefer the terms *team-based* and *factory-based*, as this better captures the functional nature of these classes of sociality. The rationale for these terms is explained below. In general, this terminology does not saddle the discussion with the incorrect notion that some groups are simple, or primitive, while others are complex and advanced (Johnson and Linksvayer, 2010, Linksvayer and Johnson, 2019). The colonies in the different classes of eusociality are simply organized differently. Whatever one chooses to call these classes is secondary. It is important to be familiar with them in order to understand how honey bees differ from other social bees.

The smallest societies of bees, sometimes called incipiently eusocial, are composed of a mother and a handful of her daughters (Danforth, 2002, Schwarz et al., 2007). These bees have a life cycle similar to that of solitary bees. The main difference is that the mother is still alive when her young emerge, and the daughters often stay at the nest to help rear their sisters (Wcislo et al., 1993, Schwarz et al., 2007). This is an example of the widely studied phenomenon of alternative reproductive tactics, as a newly emerged female can either leave and found her own nest or stay and help (Brockmann and Taborsky, 2008). The helpers in these groups are mated and fully capable of taking over from the mother as the main egg layer. These bees may thus better be called "cooperative breeders," as this is the term used to describe the same (and widely studied) phenomenon in birds (Johnson and Linksvayer, 2010, Danforth et al., 2019). Cooperative breeding in bees has been the subject of a considerable amount of study because it represents the first steps in the evolution of social behavior (Wcislo and Danforth, 1997, Rehan and Toth, 2015). It is thought that understanding these bees can shed light on the forces that select for social behavior in general (Saleh and Ramirez, 2019, Kapheim et al., 2015a, Rehan and Toth, 2015, Linksvayer and Johnson, 2019). Many species of bees in both the Apidae and the Halictidae exhibit this form of sociality. Allodapine bees, and members of the genera *Lasioglossum* and *Megalopta*, have received the most experimental attention.

The team-based societies, historically called primitively eusocial, are represented by the bumble bees and some halictids (Heinrich, 2004, Cameron et al., 2007). We refer to these groups as team-like because in these colonies bees work together as a team, rather than like cogs in a machine, as in the factory-like societies to be discussed later. In team-like societies, colonies are founded by a solitary queen in the spring. The colony then grows to a peak size of about a couple hundred bees, at which point a switch from the production of workers to the production of new queens and males occurs. The colony is annual and dies at the end of the season. The sexuals (either males, females, or both) then overwinter in the ground, to start the process again in the spring. These colonies have simple patterns of division of labor and coordinate their activities with multimodal communication signals involving sound, stereotyped movement, and pheromones (Jandt and Dornhaus, 2009, 2014). The signals are not as complex as one sees in the honey or stingless bees, however.

The factory-based societies (historically called advanced eusocial), are so called because they make extensive use of assembly lines, and are large perennial colonies that reproduce (in the bees and wasps, though not typically in the ants) by splitting in two in a process called swarming (Wilson, 1971, Johnson and Linksvayer, 2010). In these colonies, the queens and workers are morphologically distinct, something that clearly distinguishes them from the team-based societies. These societies typically have thousands of workers whose organization is characterized by a complex system of age-based division of labor. A few species, in the stingless bees, have incipient physical castes (Gruter et al., 2012). Group-level coordination of activity is achieved via a system of communication so complex it is best thought of as "social physiology" (Seeley, 1995). The honey bee falls into this category, as do the speciose pantropical stingless bees. While honey bees have received considerably more study than stingless bees, there is no reason to suspect that their colonies are more complex.

The Honey Bees

Based on life history, the 11 recognized species of honey bees fall into three classes (Michener, 1974, Hepburn and Radloff, 1998, Arias and Sheppard, 2005, Raffiudin and Crozier, 2007, Lo et al., 2010). These are the cavity nesting honey bees (eastern and western honey bees), the dwarf honey bees, and

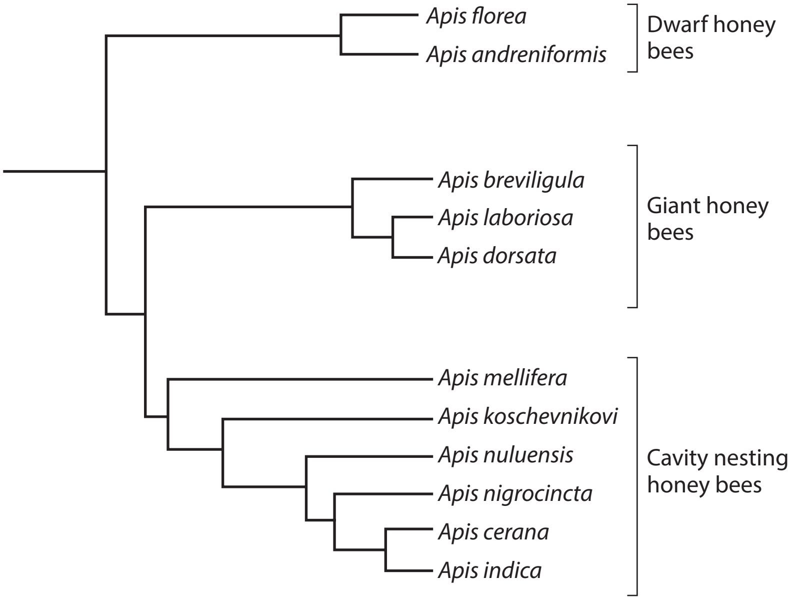
Apis florea
Apis andreniformis
Dwarf honey bees
Apis breviligula
Apis laboriosa
Apis dorsata
Giant honey bees
Apis mellifera
Apis koschevnikovi
Apis nuluensis
Apis nigrocincta
Apis cerana
Apis indica
Cavity nesting honey bees

FIGURE 2.4. Phylogeny of the genus *Apis* (*redrawn from Lo et al., 2010*).

the giant honey bees (figure 2.4). Here we focus on the characteristics that differentiate each group from the western honey bee, but in general, all honey bee species are quite similar in their biology.

There are currently two species of dwarf honey bees, *Apis florea* and *Apis andreniformis* (Raffiudin and Crozier, 2007, Lo et al., 2010, Oldroyd and Wongsiri, 2006). Their common name comes from the fact that they are considerably smaller than other honey bees. Their colonies are also the smallest, containing on the order of a few thousand bees at maturity. The two species occur in sympatry in some of their range but have mostly nonoverlapping distributions. Dwarf honey bees produce a single comb, and their nest is made in the open on the branch of a tree or shrub. The only significant physical differences between the two dwarf species pertain to coloration (*A. andreniformis* is darker) and some minor morphological differences in the proboscis and a few other structures (Oldroyd and Wongsiri, 2006). With respect to behavior, only minor differences have been shown. As for maintaining reproductive isolation between the two species when in sympatry, their mating flights occur at different times of the day (Wongsiri et al., 1997).

The giant honey bees are currently broken into four species (Lo et al., 2010). The most widespread species, *Apis dorsata*, has a range covering most of southern and Southeast Asia and parts of Oceania, while the others have limited distributions. *Apis laboriosa* occurs in the foothills of the Himalayas, while the two most recently recognized species, *Apis breviligula* and *Apis indica,* are limited to the Philippines and to southern India, respectively. In some respects, giant honey bees have a biology similar to that of dwarf honey bees. They produce a single exposed comb, for example. There are, however, some striking differences between the dwarf and giant honey bees. Giant honey bee nests, for example, are quite large and populous and tend to be clustered in the same tree (Oldroyd and Wongsiri, 2006). *A. dorsata* and *A. laboriosa* also migrate each year to take advantage of variable resources (Woyke et al., 2012). As in the case of the two dwarf species, the giant species, relative to one another, have minor differences in coloration and morphology and likely exhibit slight behavioral differences.

The cavity nesting Asian honey bee has a vast range, covering most of Asia along with the larger island chains in the Pacific, and is currently broken up into five species: *Apis koschevnikovi, Apis cerana, Apis nigrocincta, Apis nuluensis*, and *Apis indica* (Lo et al., 2010). These bees have similar biology, although genetic divergence shows that they have speciated, probably due to geographic isolation. These are bees with biology almost identical to that of *A. mellifera,* the common western honey bee, which is the subject of this book. The only major difference is that *A. cerana* has smaller colonies and smaller individual workers than *A. mellifera*. This difference makes keeping *A. cerana* less economically favorable than *A. mellifera,* which stores more honey, and has led to *A. mellifera* being imported into most of the range of *A. cerana*. Although there are many minor morphological and behavioral differences between *A. cerana* and *A. mellifera,* a considerable amount of research shows that with respect to division of labor, the waggle dance, and most other well-understood social behaviors, the two species are quite similar (Oldroyd and Wongsiri, 2006).

The Western Honey Bee, *Apis mellifera*

The western honey bee, *A. mellifera,* has a vast range that covers all of Africa, Europe, and the Middle East (Winston, 1991, Seeley, 1985a). Given that Africa is much larger than Europe, *A. mellifera* is primarily a tropical species, or rather a species equally at home in the tropics or the temperate zone. This is why the common name, European honey bee, is inappropriate. We cover the differences between the temperate and tropical subspecies of *A. mellifera* in chapter 16, but for now we simply point out that the biology of the species across its range is quite similar. Division of labor, reproductive biology, communication systems, and so forth do not qualitatively vary across the range (with a few exceptions). There are, however, strong quantitative differences between subspecies (particularly those in the temperate versus tropical zones). The most famous difference illustrates this as the defensive system is the same for all subspecies, but tropical honey bees have lower thresholds for exhibiting the same defensive behaviors (Breed et al., 2004a).

The biology of the western honey bee is the subject of this book, so we limit ourselves in this chapter to some taxonomic and interesting evolutionary information. Specifically, we address three questions: How many subspecies of western honey bee are there, where did these bees originate, and is the western honey bee domesticated? The first two questions have been the subject of much research, while the third is commonly believed although the evidence for it is debatable. Finally, from this point forward in the book, when we use the term *honey bee* or *bee*, we mean *A. mellifera* unless otherwise noted.

Apis mellifera *Subspecies*

Figure 2.5 and table 2.1 show 33 honey bee subspecies along with their geographical ranges (Engel and Schultz, 1997, Hepburn and Radloff, 1998, Engel, 1999, Ilyasov et al., 2020). These subspecies have been further grouped into four clades based on genetic similarity (Whitfield, 2006b, Han et al., 2012). These correspond to two groups with recent origins in Europe (M for western and E for eastern European bees) and one each for bees stemming from the Middle East (O) and Africa (A). In general, many of the subspecies map onto ecologically distinct habitats, meaning that local adaptation to savannas, rain forests, deserts, the Far North, the Mediterranean, and so forth is what likely caused the large number of extant honey bee subspecies. The honey bee thus has great intraspecific variation and displays quite elaborate local adaptation across its range. These traits make the species an ideal candidate for microevolutionary studies, although this subject has been somewhat neglected (but see chapter 9).

There are some particularly interesting subspecies in Africa deserving of mention (Hepburn and Radloff, 1998). The bee native to Egypt, *A. m. lamarckii*, produces hundreds of new queens when they swarm, for unknown reasons. The bee that lives on the mountaintops in East Africa, *A. m. monticola*, is a large dark bee that is thought to be gentler than the bees in surrounding areas. This may be indicative of some degree of convergent evolution between

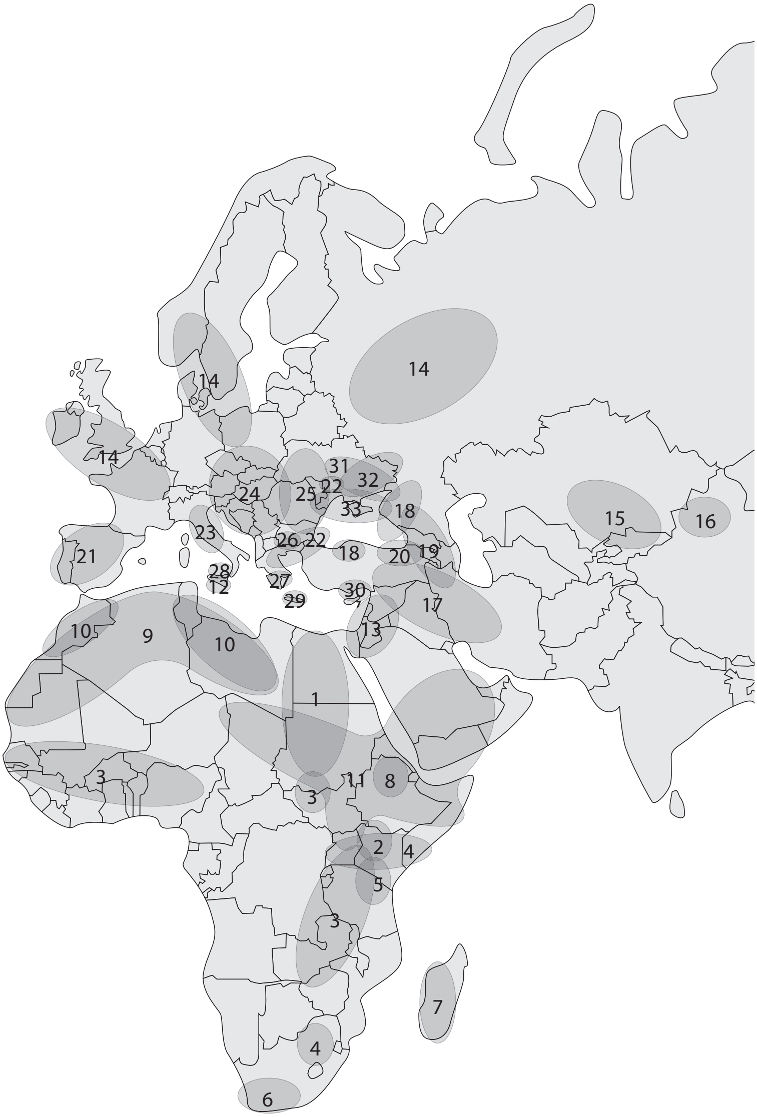
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FIGURE 2.5. Geographical range of subspecies of *Apis mellifera*. Names of subspecies are in table 2.1 (*redrawn from Ilyasov et al., 2020*).

Table 2.1. Western honey bee subspecies

Subspecies	Common name	Distribution
Africa		
Apis mellifera lamarckii	Egyptian honey bee	Egypt, Sudan
Apis mellifera litorea	East African coastal honey bee	East African coast
Apis mellifera adansonii	West African honey bee	West Africa
Apis mellifera scutellata	African savanna honey bee	East, central, and southern Africa
Apis mellifera monticola	African mountain honey bee	Mountaintops of East Africa
Apis mellifera capensis	Cape honey bee	Cape region of South Africa
Apis mellifera unicolor	Madagascar honey bee	Madagascar
Apis mellifera simensis	Ethiopian honey bee	Ethiopia
Apis mellifera sahariensis	Saharan honey bee	Sahara
Apis mellifera intermissa	Tellian honey bee	Morocco, Libya, Tunisia
Apis mellifera jemenitica	Arabian honey bee	Arabian Peninsula, Horn of Africa, Sudan, Chad
Western Asia and Middle East		
Apis mellifera ruttneri	Maltese honey bee	Malta
Apis mellifera syriaca	Syrian honey bee	Syria and neighboring countries
Apis mellifera mellifera	German honey bee	Northern Europe
Apis mellifera pomonella	Tian Shan honey bee	Tian Shan mountains
Apis mellifera sinisxinyuan	Xinyuan honey bee	Uygur autonomous region of China
Apis mellifera meda	Persian honey bee	Iran, Iraq, Syria, Turkey
Apis mellifera caucasia	Caucasian honey bee	Southern Russia, Turkey, Georgia
Apis mellifera remipes	Armenian honey bee	Southern Russia, Armenia, Iran, Georgia
Apis mellifera anatoliaca	Anatolian honey bee	Turkey, Iran, Armenia, Syria
Europe		
Apis mellifera iberiensis	Spanish honey bee	Spain, Portugal
Apis mellifera macedonica	Macedonian honey bee	Bulgaria, Greece, Macedonia, Ukraine
Apis mellifera lingustica	Italian honey bee	Italy
Apis mellifera carnica	Carniolan honey bee	Much of eastern Europe
Apis mellifera carpathica	Carpathian honey bee	Ukraine, Bulgaria, Romania, Moldova
Apis mellifera rodopica	Bulgarian honey bee	Bulgaria
Apis mellifera cecropia	Greek honey bee	Greece
Apis mellifera siciliana	Sicilian honey bee	Sicily
Apis mellifera adami	Cretan honey bee	Crete
Apis mellifera cypria	Cyprian honey bee	Cyprus
Apis mellifera artemisia	Russian steppe honey bee	South Russia, Ukraine
Apis mellifera sossimai	Ukrainian honey bee	Ukraine, Crimea, southern Russia
Apis mellifera taurica	Crimean honey bee	Ukraine, Crimea, southern Russia

Source: Ilyasov et al., 2020.

monticola and subspecies in the temperate zone, as both inhabit cooler climates. Finally, the Cape honey bee, *A. m. capensis,* is capable of parthenogenetic reproduction.

Apis mellifera *outside Its Native Range*

The native range of *A. mellifera* is vast, and the current range extends to everywhere human beings live. The origin of the honey bees invasive to several parts of the world are complex, as considerable hybridization between many subspecies has occurred in the introduced range over the last several centuries. We briefly review the history of the bees in North America, but similar stories could be told for the large populations of honey bees in Australia, China, Japan, Argentina, and so forth.

Most of the honey bees of North America are a mixture of bees of many European subspecies (Sheppard, 1989a, 1989b, Harpur et al., 2012, 2015). Early imports were of the northern race, *A. m. mellifera,* sometimes called the German black bee. These bees were brought to New England from England and quickly became invasive across the United States and Canada. The northern bee, however, is quite defensive and difficult to work with. Initially, this was not a problem, but it became one with the invention of modern beekeeping practices, which involve more manipulation of colonies than the older skep hive methods. Later introductions of bees thus favored the Italian subspecies, *A. m. lingustica,* a gentler bee. Most American bees are now of this stock (Kritsky, 1991, Schiff et al., 1994). Bees have also been imported recently from various parts of eastern Europe (Cobey et al., 2012). Finally, Africanized bees are established across much of the southwestern United States, all of Central America, and most of South America, and are essentially a wild invasive species (Schneider et al., 2004b). These bees have their origin in southern Africa but have strong introgression of European DNA (Rinderer et al., 1991, Sheppard et al., 1999, Pinto et al., 2005, Rangel et al., 2016b).

The Place of Origin of Apis mellifera

The origin of *Apis mellifera* has been the subject of considerable debate (Wilson, 1971, Ruttner et al., 1978, Garnery et al., 1992, Whitfield et al., 2006a, Han et al., 2012). The split from other cavity nesting bees is thought to have occurred six to nine million years ago, and the split between the four extant groups (A, M, E, O) is thought to be about one million years old (Cornuet

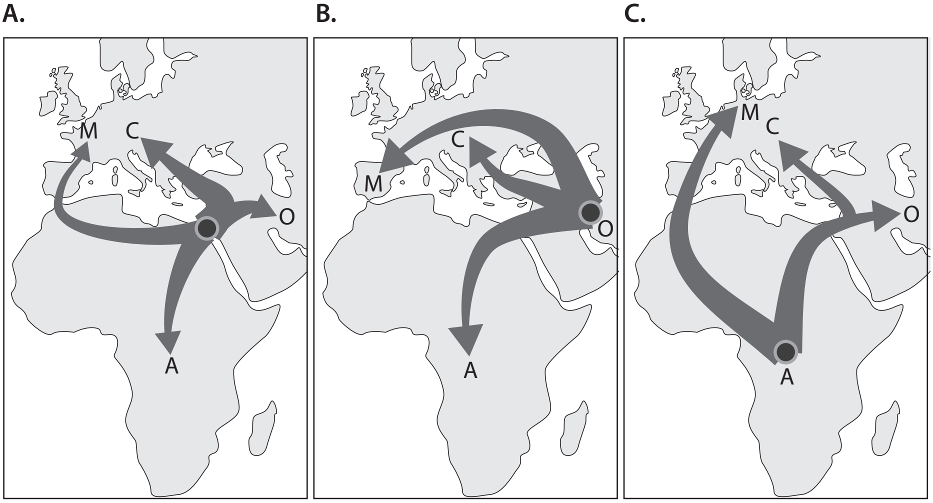


FIGURE 2.6. Models for the origin of *Apis mellifera*. (A) The hypothesis of Ruttner (1978); (B) the hypothesis of Cornuet and Garnery (1991); and (C) the path suggested by the work of Whitfield et al. (2006a) (*adapted from Han et al., 2012*).

et al., 1991, Arias and Sheppard, 2005). The contention has involved where these splits took place and the nature of the subsequent spread of the species over its vast range (Whitfield et al., 2006a, Han et al., 2012). At least three ideas have been put forward (figure 2.6). Ruttner et al. (1978), based on a morphometric analysis, proposed that the species arose in the Middle East, or East Africa, and then spread into Africa and Europe via two routes. Another group proposed an origin in the Middle East, based on an analysis of mitochondrial DNA, with radiations into Africa and Europe, but with the difference that the M and C clades both derived from the O clade (Cornuet et al., 1991). Finally, Whitfield et al. (2006b) used genomic data, over 1000 single nucleotide polymorphisms (SNPs) from bees across the range, to provide support for the idea that the species arose in Africa and then spread in much the same manner as proposed by Ruttner et al. (1978). Han et al. (2012) reanalyzed the data of Whitfield et al. (2006a) by relaxing various assumptions and removing potentially problematic data relating to bees that may be hybrids. They found that, under a variety of realistic assumptions, the data do not allow one to distinguish between the competing hypotheses. Finally, the most recent study used mitochondrial genomes from most subspecies to support the original notion of Ruttner, that is, a northern African or Middle

Eastern origin with two northern routes into Europe (Tihelka et al., 2020). In general, all extant genomic studies suffer from sampling problems, in that there are usually more samples from Europe than Africa, and parts of the Middle East are poorly sampled. Further, even if sampling were grossly equal between Europe and Africa, this would still be wildly out of proportion considering the relative areas. The Congo alone is one-quarter the size of Europe, so comparing a few samples from there with a few samples each from several European nations makes little statistical sense.

Are Honey Bees Domesticated?

Honey bees and silkworms are often referred to as the only domesticated insects (Seeley, 2019, Zhou et al., 2020). For the silkworm, domestication is clear, as the males cannot fly, and the species is quite derived relative to its wild ancestor. For the honey bee, however, it is not so straightforward. It is important to explore this issue because whether it is best to think about the honey bee as wild, managed, domesticated, or some complex combination of all three sets the stage for how we think about the honey bee as a model for several questions in biology. If they are managed only, then the honey bee can serve as a model for many basic questions in organismal biology. However, if honey bees are strongly domesticated, like cows, then their utility for basic evolutionary questions might be diminished.

Are There Still Significant Wild Populations of Apis mellifera*?*

The main problem with viewing the honey bees as a domesticated species is that the species is wild over most of its native range (Hepburn and Radloff, 1998). As stressed earlier, the western honey bee's native range is Africa, Europe, and the Middle East. Africa is many times larger than Europe and the Middle East, and modern beekeeping (the context in which artificial selection could occur) is rare there outside of South Africa. Honey collecting from wild bees is common in Africa, of course, but this is no different than hunting, and hunting an organism does not lead to its domestication. Hence, honey bees are wild over most of their native range, not feral, since they were never domesticated in the tropics. Feral, of course, refers to a domesticated animal that has returned to the wild.

The question is more complex in Europe, but the data suggest a picture quite different from the commonly believed (but never shown) idea that wild

A. mellifera was wiped out by domestication and mites. We cover this issue in depth in chapter 10 in the section on population biology, but for our purposes here, the wild populations of *A. mellifera* in many parts of Europe are healthy and not yet impacted greatly by managed bees (Pinto et al., 2014, Groeneveld et al., 2020). Of course, there are regions of Europe where this is not true.

Selective Breeding of Honey Bees

The most powerful tool for the animal breeder is selective breeding, the pairing up of males and females with desired traits. It is quite difficult to selectively breed honey bees, however. The males and females mate in what are called drone congregation areas far from the hive (Gary, 1962). There, virgin queens mate with an average of 12 males from colonies spread out over a large area (Tarpy and Nielsen, 2002). Hence, pairing up desirable females and males for mating was exceedingly difficult until the 1940s when artificial insemination techniques were finally realized (Laidlaw, 1944). Artificial insemination has recently become widely used in the United States by queen breeders (Cobey et al., 2012), but historically it was not widely used. Its main historical use was in behavioral genetics studies.

In spite of the difficulty of breeding bees, some designer bees were selectively bred with great effort. The Buckfast bees, for example, were carefully bred in geographically isolated apiaries in which nondesirable males would be sure to be absent. They are advertised as being gentle and productive. These bees could accurately be called domesticated (Crane, 1999). In general, however, such domesticated bees were not commonly used anywhere in the world, as they were costly, and it was always cheaper to just use whatever honey bees (wild or invasive) were locally available.

Artificial Selection on Honey Bees

Although selective breeding of honey bees was difficult prior to the 1940s, the belief that beekeepers have always selected for and against traits by culling aggressive bees or favoring productive colonies is common. It is impossible to ascertain the accuracy of this belief, however. While one can find examples of this occurring in a sophisticated manner in the distant past (Crane, 1999, Seeley, 2019), this was probably only for a small minority of the temperate zone honey bee population. In general, until the advent of the Langstroth beehive, beekeeping was done in a manner in which colony manipulation was quite difficult. Colonies were established in spring (usually swarms were captured), but were then left alone until harvest time, at which point the colony was often destroyed, as the harvest was so invasive it triggered absconding. Thus, historically, beekeepers did not interact much with their bees. The temperament of the bees was thus of little importance. The amount of honey collected was important, of course, but given the methods for colony founding and honey collection, there was little opportunity for selection.

In the mid-1800s, Langstroth (1857) developed the beekeeping hive currently in use, which contains removable frames. This hive allows one to inspect and manipulate a colony without seriously damaging or destroying it. Once this hive was widely adopted, the opportunity to select against highly defensive bees and for those that produce a lot of honey was possible, and it undoubtedly has occurred (Nolan, 1929, Cobey et al., 2012). For queen breeders, this process has been explicit. It is difficult to know how much this selection has changed bees in regions where the Langstroth hive has been in widespread use. It is clear that there have been no genetic bottlenecks of the sort associated with domestication (Wallberg et al., 2014). Further, it is often said that the German bee is as defensive as ever, but as for many questions in bee biology, there is little beyond hearsay in terms of data. What is clear is that some degree of selection for honey production, survivorship, and health in general, and against defensiveness has occurred in European honey bees. Given that commercial beekeepers care immeasurably more about honey production and colony strength than they do about defensiveness, selection for such economically vital traits have probably been the primary targets of selection.

Management versus Domestication

To return to the central question, most of the bees kept by beekeepers have been selected to a degree for honey production and perhaps gentleness. Otherwise, they are much like wild bees. The clearest indication of this is that one can still go anywhere in the world and catch wild bees (even in Africa), put them into modern hives, and use modern beekeeping practices successfully. This would be equivalent to capturing wolf puppies, treating them like dogs, and having them grow up to act like dogs. Of course, this does not occur with wolves, which is why they are illegal to keep in so many places. In fact, replacing domesticated animals with their wild relatives and using standard farming practices would be a disaster in most cases, but it works for honey bees (De Jong, 1996). This brings us to the difference between management and domestication.

Asian elephants are not domesticated, although they can be observed working alongside humans in many Asian countries. Essentially, they are captured as babies, or reared from birth from captive elephants, and trained with great skill over many years. This is a process of management, not domestication. The same can be said for honey bees. Beekeepers have not changed their bees in any fundamental way; rather, they have learned over many years how to manage them. The smoke we use, for example, short-circuits the attack response of bees. The attack response is still there, but we have simply learned how to quell it. Likewise, bees reject foreign queens, which makes queen replacement—a desirable thing for the beekeeper, but not the bees—quite difficult. However, by putting a new queen into a cage that allows her to absorb the new hives' odor from a safe position before introduction, we can replace queens. Again, we have not changed the bees' nestmate recognition system, we have rather learned how to work around it. Being an expert beekeeper is to have mastered these techniques for working around the basic biology of the honey bee. Hence, even though some bees clearly have been artificially selected so much so that they can be called domesticated, domestication is not how beekeeping works. Beekeeping is about management of bees, and it works well for domesticated or wild bees.

Managed versus Wild Bees When in Sympatry

The ratio of wild to managed or domesticated bees in a place like Germany or New York is simply unknown. It likely varies from mostly managed in some places to mostly wild or invasive in others. What is unfortunate, however, is that even in places where *A. mellifera* is a native pollinator of great importance, like Britain, one still hears the belief expressed, even by scientists, that honey bees are domesticated invasive species that exist in opposition with native bees. This usually comes up in discussions about helping native pollinators. Essentially, *some* honey bees have been domesticated, and now the convention seems to be that *all* honey bees are domesticated and somehow unnatural, even in places where they are indigenous and probably the most abundant native pollinators.

The Verdict?

To sum up, there is no simple answer to the question of whether honey bees are domesticated. In this book, we prefer not to think of them as domesticated animals, based on the evidence, but some bee scientists disagree. This section has tried to present both sides of the issue. Perhaps a compromise is to say that there are clearly many wild *A. mellifera* honey bees in the world both as native and invasive species (African and Africanized bees in particular). There are also quite a few domesticated honey bees. Most honey bees that are managed fall into a gray area in that they have experienced selection as a result of beekeeping, but how significant this artificial selection has been is difficult to say. Given the ease with which these bees go feral, it seems unlikely to have been profound. Finally, a key point to keep in mind when pondering this issue is that the practice of beekeeping is not about selecting bees for desirable traits and getting rid of undesirable ones. Rather, it is about learning enough about bee behavior, physiology, and so forth to manage them for the benefit of people, and the resulting encyclopedia of beekeeping knowledge works well for wild or domesticated honey bees.

3

Development

Developmental biology explores the mechanisms of growth and differentiation. Traditional topics dealing with insects include embryogenesis, sex determination, and metamorphosis, all of which have been well studied in the honey bee. We organize our review of this body of work in a straightforward way. Honey bees are holometabolous insects with four life history stages: egg, larva, pupa, and adult (plate 1). We start with the egg and work our way through to adulthood. For each life history stage, we begin with a description of the phenotypes and any relevant organismal biology. We then go into detail about what is known at the physiological and molecular genetic levels. Whenever it seems necessary to understand the work to come, we take a step back from the bees to introduce the reader to the relevant core ideas, typically based on work in the fly model system.

Eggs

Honey bee eggs are whitish, or somewhat translucent, and sausage shaped (plate 2). Fertilized eggs become female, while unfertilized eggs become male, a phenomenon called haplodiploidy (Beye, 2004). Female eggs can be reared either as workers or queens depending on colony needs. Bees of each form are reared in different types of cells (Winston, 1991). Worker cells are the smallest; they are hexagonal and quite consistent in size. Queen cells, or cups, are oblong and hang from the bottom of the comb (plate 3). There are often a few empty queen cups present at any given time, but in general they are prepared just before queen rearing begins and torn down after queen production ends. Eggs destined to be male are laid in drone comb, which is like worker comb but larger.

The queen typically lays all the eggs in a nest. Tests for eggs of worker derivation in queenright colonies show very low, but consistent, rates of worker laying (Ratnieks, 1993). Worker-laid eggs are quickly eaten, however, by the other workers in queenright colonies (Ratnieks, 1995). Worker-laid eggs are allowed to develop when the colony becomes permanently queenless (Page and Erickson, 1988). These issues are covered in the chapters on reproduction (chapter 8) and evolution (chapter 9). With respect to laying behavior, the queen inspects the cell she is about to lay the egg in, presumably to determine whether to fertilize the egg. She appears to use her forelegs to measure the cell to make this decision (Koeniger, 1970). Healthy queens lay a single egg per cell. The eggs are glued upright in the cell, but fall over slowly before hatching. Worker-laid eggs are smaller than queen-laid eggs, positioned differently, and often somewhat misshapen (Taber and Roberts, 1963). Many of them can also be laid in a single cell, and they are glued to the side of the cell since the worker's abdomen cannot reach the bottom of the cell.

Early development in most insects occurs in what is called a multinucleate syncytium (Cridge et al., 2017). This is to say that the early cell cleavages are not complete. Early in development, one large cell with many nuclei forms as incomplete divisions occur. This syncytial embryo is regionalized by the expression of genes from localized nuclei and then undergoes cellularization, whereby cell membrane grows and partitions off each nucleus. Gastrulation follows and hatching of the egg takes three days, although this varies with temperature and genotype (Harbo et al., 1981). The actual hatching event is nearly impossible to observe because the egg slowly disintegrates, leaving a tiny larva floating on top of brood food (DuPraw, 1961). This is in contrast to what occurs in most insects, in which the hatching process is somewhat reminiscent of a reptile egg hatching, with the emergence of the young leaving behind a pierced shell.

Embryogenesis

Embryogenesis is the process through which an undifferentiated egg becomes a structured multicellular organism through a process of growth and differentiation. We start with a few words about the core principles and then transition to work on bees. This section is shorter than it could be given the amount of data collected to date, but since honey bee early development only differs from that of the model insect in key ways, we focus on those aspects of divergence and neglect the many conserved aspects for which the reader could consult a standard reference.

Our general picture of insect early development is mainly based on studies in *Drosophila melanogaster* (Ingham, 1988, Lawrence and Morata, 1994). We review this fly work for comparative purposes before turning to what occurs in bees. Essentially, the spatial information present in the developing organism starts simply and gets more complex as development proceeds. Earliest development occurs in four phases, characterized by different classes of genes (Mallo and Alonso, 2013). Prior to fertilization, the egg already contains maternally produced mRNAs that form concentration gradients across the cell, providing the information necessary to form anterior-posterior and dorsoventral axes. Following fertilization, gap genes interact with the maternally derived genes to spatially localize their own expression in order to map out the head, thorax, and abdominal areas. Then pair rule genes are expressed, which interact with the gap genes and each other to produce stripes of their own expression across the cell. Finally, segmentation genes are expressed that use the information generated thus far, along with interactions with one another, to outline the regions that will form the segments of the larva. In addition to this process leading to segmentation, the germ line segregates early through the formation of pole cells at the posterior of the embryo. These cells ultimately become the gonads. There are many other core aspects of early development, of course, but these represent those relevant for our discussion.

Honey Bee Embryogenesis

Work in honey bee embryogenesis to date has largely focused on determining how much of the now canonical picture of early development from *Drosophila* is true for bees (Cridge et al., 2017). This serves two functions. First, it explores how good a model *Drosophila* is for other insects. Second, it sheds light on the idiosyncratic nature of bee development. Taxonomically restricted variation may sometimes be of little interest to the basic developmental biologist, but it is vital to the specialist. Below we cover the process of embryogenesis in bees, as contrasted with what occurs in *Drosophila*.

EARLY DEVELOPMENT

For *Drosophila,* the four classes of genes previously discussed are well understood, and it was assumed by early researchers that their expression would be conserved across insects (Carroll et al., 1995, Raff, 2000). One of the first studies to make use of the honey bee genome thus sought to determine what percentage of key developmental genes, in these and other pathways, were conserved between flies and bees (Dearden et al., 2006). The result was that, although there is strong conservation across most gene families, some genes are less conserved than others, especially those that act earliest in development. Further, even in cases in which the genes are conserved, their functions may not be. An overview of some major gene families and a few case histories of developmental patterns in bees versus flies illustrate these conclusions.

There are many canonical gene families involved in pattern formation, such as the Wnt, Hox, Notch, and Dpp families (Pires-daSilva and Sommer, 2003). We use the Hox genes as an example. Hox genes are transcription factors that bind DNA via the Hox protein domain. Hox genes are the most famous developmental genes, as they were found to be nearly universally conserved across the Metazoa, leading to a paradigm shift in evolutionary biology that stresses genetic conservation rather than unique evolutionary genetic paths through time (Carroll et al., 1995, Carroll, 1995).

Figure 3.1 shows conservation within the Hox complex in fruit flies and honey bees. There is remarkable conservation in terms of presence and absence and order along the chromosome. There are some missing genes, however. Bicoid (*bcd*), for example, a key early pattern formation gene in fruit flies, is missing in bees (and many other insects). What are we to make of this? If you think of these genes as analogous (in a metaphorical sense) to load-bearing elements in a structure, then missing any of them is shocking. If, in contrast, you think of them as important genes that serve functions that, in principle, could be performed by other genes, then missing one or two is not surprising. In short, early work supposed these genes to be like load-bearing elements, while current studies suggest the latter interpretation. This is because whenever a comparison is made between distantly related insects for genes in a core pathway, there are always some genes that were thought to be essential that are missing in some groups.

With respect to presence or absence, figure 3.1 shows that there is remarkable, though not complete, conservation of the major genes important in early development. How about in patterns of expression and function? The same genes might be there, but they might play different roles across systems. There are three basic results that have emerged from studies of this question. First, there are cases where the process of development at the molecular level is nearly completely conserved. Second, there are cases where the basic process seems conserved, but key players are missing. In these cases, further work

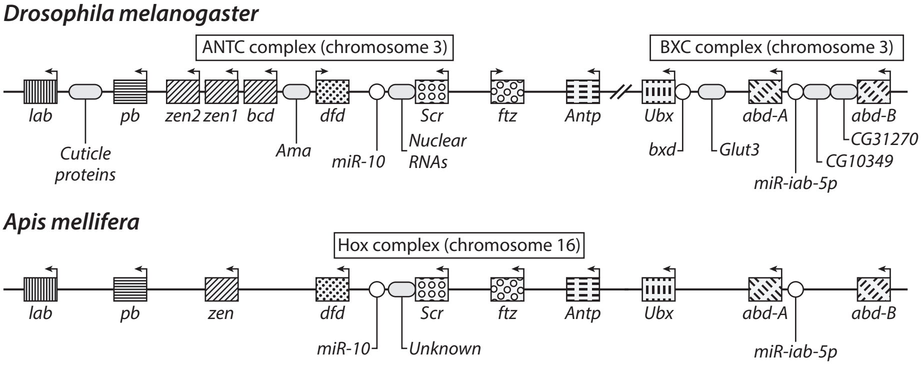

Drosophila melanogaster
ANTC complex (chromosome 3)
BXC complex (chromosome 3)
lab
pb
zen2
zen1
bcd
dfd
Scr
ftz
Antp
Ubx
abd-A
abd-B
Cuticle proteins
Ama
miR-10
Nuclear RNAs
bxd
Glut3
miR-iab-5p
CG10349
CG31270
Apis mellifera
Hox complex (chromosome 16)
lab
pb
zen
dfd
Scr
ftz
Antp
Ubx
abd-A
abd-B
miR-10
Unknown
miR-iab-5p

FIGURE 3.1. Conservation of the Hox gene family between *Drosophila* and the honey bee (*redrawn from Dearden et al., 2006*).

typically shows that the missing gene(s) are being filled in by other gene(s). Essentially, some molecular actors have changed, but the pathway and its network structure is kept largely intact. Third, there are cases where fly and bee development differ in some fundamental way. In these cases, it is rarely—perhaps never—the case that the bee pattern is unique; rather, it is that the insects as a whole are broken up into a few groups with respect to this trait and bees and flies are in different classes. The first case is obvious, so we give examples of outcomes two and three.

As mentioned, *bcd* is missing in the bee genome. This gene is one of the maternally expressed genes that produces a gradient in the egg, the simplest mechanism allowing for spatial positioning (Berleth et al., 1988). As mentioned previously, early development is broken into four stages that play out sequentially. Work in other insect systems shows that the maternally expressed genes, like *bcd,* are the least conserved of any across the four stages (Bucher and Klingler, 2004, Mito et al., 2006, Dearden et al., 2006). Indeed, *bcd,* a crucial early-acting gene in *Drosophila,* is only present in some fly species and absent from most insects. A good question is thus, How does the novelty of the early process ultimately fit into downstream conserved pathways?

Wilson et al. (2010) explored this question by looking at the expression of the bee gap genes. The gap genes (*Krűppel, Giant,* and *Caudal*) fall in between the earliest maternally derived genes, like *bcd,* and the later pair rule genes, which are more similar between bees and flies than are genes in the classes expressed earlier. Recording gap gene expression across the embryo and conducting RNA interference (RNAi) studies knocking down expression of each gap gene established that the three gap genes have mostly conserved functions in bees and flies. *Giant* is involved in mapping out the head, *Krűppel* the thorax, and *Caudal* the abdomen (in gross terms), but the functions of each gene appear to be more extensive in bees than in flies. *Giant* and *Caudal* especially are maternally produced, in addition to being endogenously expressed in the embryo, and have different expression levels in the honey bee relative to fruit fly eggs. The preliminary conclusion drawn from this work is that the gap genes likely do the work of the missing gene *bcd* in bees in addition to the work they do in flies.

This gap gene story is a good example of how tweaking the behavior of some aspects of the system can allow for strong overall conservation, but with significant variation between systems. There are several other examples in honey bees. *Tailless,* for example, is a nuclear receptor important for determining the anterior-posterior axis in flies (Pignoni et al., 1990). Comparative genomics showed that the torso signaling pathway, which controls *tailless* expression in *Drosophila,* is missing in bees (Dearden et al., 2006). Wilson and Dearden (2009), however, showed that the pattern of expression of *tailless* and its function is nevertheless conserved. It is still unclear how *tailless* expression is controlled in bees, but preliminary evidence suggests a difference in posttranscriptional regulation. The take-home message, however, is that in spite of novel aspects (regulatory mechanisms in this case) conservation in function is conserved; in short, a complex pattern of conservation and novelty across the network.

Much work in this field is like what we have reviewed for *tailless* and the gap genes. Studies have looked at the pair rule genes, which differ across insect orders, and at *Pax* expression, for example, with conceptually similar results (Osborne and Dearden, 2005, Wilson and Dearden, 2011). This work is done in a phylogenetic context in which bees, along with the beetle *Tribolium,* the wasp *Nasonia,* locusts, and other insects together form a comparative group for the study of early development (Dearden, 2018). Honey bees may not be central to this field, but they have contributed some major insights and, given their unique biology, could play a larger role in the future.

DEVELOPMENTAL PROCESSES NOT CONSERVED WITH MODEL SYSTEMS

Developmental patterns that strongly differ in bees relative to fruit flies are typically associated with sexual traits. Sex determination itself is like the previously discussed developmental processes in that the novelty only pertains to the upstream part of the gene network, while the downstream elements are highly conserved. However, given the mechanism used by bees to determine sex (haplodiploidy), the mechanisms of sperm production in males and dosage compensation in this sex must differ for bees relative to diploid organisms. How dosage compensation works in male honey bees is currently not understood. With respect to sperm production, honey bees make use of a highly derived form of meiosis in which the first meiotic event is abortive, in that all chromosomes end up in one secondary spermatocyte (Lago et al., 2020). The second division is also peculiar, as it leads to two spermatids of quite different size and it is unclear if the smaller of these is also abortive. In general, drone reproductive development is quite derived, and the interested reader should consult Lago et al. (2020) for a review of past and present knowledge. Finally, several genes key to *Drosophila* sperm production via meiosis are missing in the honey bee genome, further suggesting quite derived biology (Dearden et al., 2006).

There is one other aspect of early bee development that differs strongly from the model system that has received some attention. In most insects, the germ cells separate early in development from cells destined to become somatic tissue (Warrior, 1994). These cells are called primordial germ cells (PGCs). How these cells form varies across distantly and even closely related taxa. In fruit flies, PGCs form very early in the posterior of the embryo and are called pole cells. In many other insects, a structure called the oosome forms early in embryogenesis and eventually gives rise to germ cells (Klag and Bilinski, 1993). In both cases, some of the earliest cells are segregated from the others in the classic manner in which soma and germ cells are thought of as being separate. However, some organisms, such as Orthoptera, do not form PGCs by early segregation, but later in development some cells in the right embryonic location are induced to become PGCs (Chang et al., 2002).

It has long been known that honey bee embryos do not have pole cells or oosomes (Nelson, 1918). PGCs are identifiable late in embryogenesis, however, along the dorsal surface of the abdomen. Dearden (2006) used two genes whose expression are good markers for PGCs in those insects with early PGC segregation to look for PGCs in early honey bee embryos. While some early transient expression of the markers (*vasa* and *nanos*) was found, there was no clear indication that either marker was expressed in a manner consistent with PGC segregation. His conclusion was that either PGCs are formed later in development by induction or early PGC segregation occurs in honey bees via unknown and nonconserved genetic mechanisms.

Sex Determination

Contemporary work on sex determination in honey bees follows a similar rationale to the work on other aspects of early development. Extensive work in the model system has established a conceptual paradigm for how the process is likely to work. Work in honey bees, along with that in other insects, starts with the paradigm and establishes how much of it is present in the nonmodel system. With respect to areas of nonconservation, there is then an attempt to elucidate the nature of the novelty and determine how it interacts with the more conserved aspects of the process. This is not the whole story, of course, because the study of sex determination in bees is older than in fruit flies. Nevertheless, it is a good way to view the problem since the genetic tools available for the model system has led to a greater depth of understudying in fruit flies relative to honey bees. Given this background, it is useful to begin with a brief review of how sex determination works in fruit flies and other non-haplodiploid organisms.

Fruit flies are a diploid organism with variable sex chromosomes. Sex determination depends on the dosage of what have been named X-linked signal elements, or XSEs (figure 3.2). XSEs are essentially a large number of genes spread across the X chromosome. In eggs with two X chromosomes, there are twice as many XSEs as there are in XY individuals. The proteins translated from these XSEs bind to the promoter of the Sexlethal gene (*Sxl*) and activate its expression only if they are present at high dosage. XX supplies sufficient dosage, XY does not. After this initial signaling from the XSEs, *Sxl* then drives its own expression through early development in an autocatalytic loop before directing production of a sex-specific product (via alternative splicing) from the gene transformer (*TRA*), which in conjunction with a binding partner, transformer2 protein (*TRA2*), directs the expression of the female-specific transcript of double sex (*dsx*). The transformer also inhibits the expression of fruitless (*fru*). Most sexual dimorphism between the sexes in fruit flies is controlled by *dsx*, while *fru* controls sex-specific behavior.

In males, the low dose of XSEs, from one X chromosome, leads to the expression of a male-specific transcript of *Sxl* that is nonfunctional (has a premature stop codon). Lack of a functional *Sxl* protein causes production of a *TRA* mRNA that is also nonfunctional, for the same premature stop codon reason, and the lack of a *TRA* protein leads to the default splicing of *dsx*, which in fruit flies is for the male transcript. Lack of the female-specific *dsx* transcript, which inhibits production of *fru*, leads to the expression of *fru* in XY eggs and ultimately to male behavior as well as morphology.

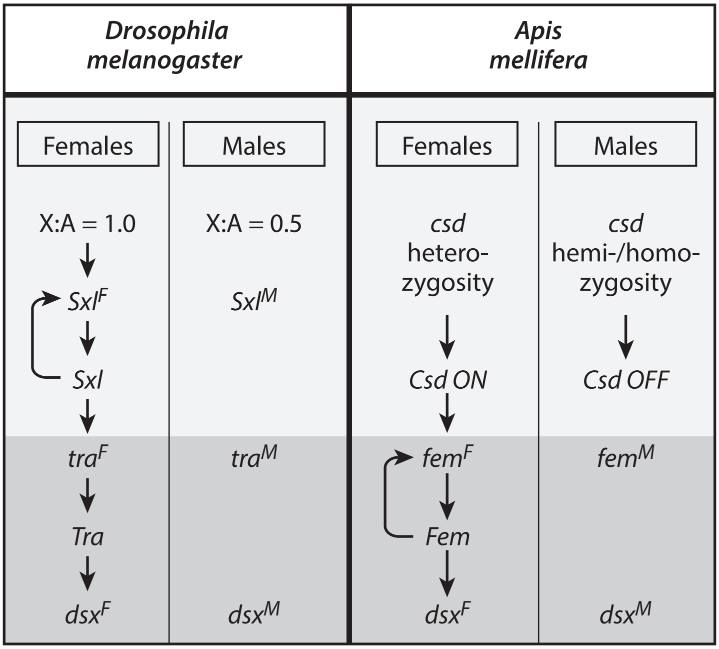

Drosophila melanogaster
Apis mellifera
Females
Males
Females
Males
X:A = 1.0
X:A = 0.5
csd heterozygosity
csd hemi-/homozygosity
SxlF
SxlM
Sxl
Csd ON
Csd OFF
traF
traM
femF
femM
Tra
Fem
dsxF
dsxM
dsxF
dsxM

FIGURE 3.2. Gene pathways controlling sex determination in *Drosophila* and the honey bee (*redrawn from Gempe and Beye, 2011*).

Considerable work has gone into exploring how much of this network is conserved in other insects (Gempe and Beye, 2011). It turns out, for example, that XSEs are uncommon. In other flies, the Y chromosome contains a gene that directly inhibits expression of the transformer, and this leads to male phenotype via an otherwise conserved network (Meise et al., 1998, Dubendorfer et al., 2002). Insects in other orders are variable in their initial triggers of male versus female development such that what has emerged is that the only absolutely conserved aspect of the network controlling sex determination is the *TRA*/*dsx* axis. All major insect lineages have sex-specific splicing of *TRA* and *dsx*, and *dsx* is the transcription factor that is central for controlling sex-specific gene expression for most downstream pathways through development and in adults.

Honey Bee Sex Determination

That male bees develop from unfertilized eggs was the first clear demonstration of a sex determination mechanism in any organism (Beye 2004). The discovery was made in 1845 by Johann Dzieron, a priest from Silesa, which is now part of Poland. The proof was simple: an unmated queen can only lay male eggs. Relatively soon after this, when cytological studies were topical in biology, it was shown that honey bee males are haploid and females (workers and queens) are diploid (Nachtsheim, 1913). Later work showed that this system of haplodiploid sex determination is not as uncommon as one might think given that some 20% of organisms have it, including all the Hymenoptera, mites, Thysanoptera, some beetles, and rotifers (Beye, 2004).

A breakthrough in the mechanism of haplodiploid sex determination came with the discovery of diploid males in a parasitoid wasp, *Bracon hepetor* (Whiting, 1933). It was shown that it is not haploidy per se that causes maleness, but some consequence of having only one allele of one or more genes. Whiting (1946) worked out, in another parasitoid wasp, *Habrobracon juglandis,* via classical genetics approaches involving inbreeding and crosses/backcrosses, that variation at one locus, named the complementary sex determination locus (*csd*), determines sex in these systems. Females are heterozygous at this locus and haploid males are typically hemizygous. Diploid males are homozygous at the sex determination locus. Diploid males are sterile, and in the honey bee are killed by the workers early in development.

The next breakthrough in this field occurred when two separate groups, one led by Greg Hunt and Rob Page (Hunt and Page, 1994, 1995) and the other by Martin Beye (Beye et al., 1994, 1996), used genetic mapping to locate the *csd* locus. Two markers segregating with the locus were found (one by each group), which were later shown to be close together. These two groups then teamed up to identify *csd* by walking molecularly along the chromosome. By fine-mapping the gene starting from a colony-level phenotype (patchy brood pattern), they found that the *csd* gene encodes an arginine-serine-rich (SR) protein, a gene class typically involved in the control of RNA splicing (Beye et al., 2003). The gene was also shown to have sequence similarity to *TRA* in *Drosophila*. This first pivotal study also used RNAi to knock out *csd*, which was found to lead to male development, indicating that male is the default program for bees.

After the discovery of *csd*, things moved rapidly. Figure 3.2 shows the bee sex determination pathway as we currently understand it (Beye et al., 2003, Hasselmann and Beye, 2004, Hasselmann et al., 2008a, Gempe et al., 2009). The initial signal for guiding sex determination is the *csd* locus. Being heterozygous at this gene, encoded by amino acid differences in an arginine-serine-rich domain and a variable-length amino acid stretch, causes the production of a female-specific transcript of the feminizer protein via a currently unknown mechanism. The feminizer (*fem*) protein was the second, and last, protein found in the region identified by genetic mapping. It resides in a tandem array with *csd*. This protein has stronger sequence similarity to *TRA* and is thought to be the bee ortholog of *TRA*. Thus, *csd* is the result of a recent gene duplication followed by rapid positive selection for neofunctionalization (Beye et al., 2003, Hasselmann and Beye, 2004). The *fem* protein was shown to be downstream from *csd* in the sex determination pathway based on RNAi studies in which one or the other was knocked out. It was also shown that *fem* did not need reinforcement from *csd* after the start of *fem* expression, as *fem* autocatalyzes its own expression through development. This was shown by injecting male embryos with *fem* transcripts (Gempe et al., 2009). As in fruit flies, the female-specific transcript of *fem* directs the expression of the female-specific transcript of *dsx*. For males, lack of complementarity at the *csd* locus leads to lack of functionality of *csd*, which causes the default (nonfunctional) male transcript of *fem* to be expressed. This leads to the default expression in *dsx*, which is for the male isoform. The first female mutants in honey bees that were generated for the *dsx* gene using the CRISPR/Cas9 method showed that *dsx* plays a role in the development of sexual development of reproductive organs, but not in the sexually dimorphic traits of the head (Roth et al., 2019).

This work on sex determination in flies and honey bees illustrates wonderfully how a mosaic of novelty and conservation characterizes developmental gene networks across large taxonomic distances. This is a trademark of the field of evo-devo, into which these bee data fit nicely (Carroll, 2008). The take-home message from this work might well be to expect conservation with the model system when beginning the study of some trait in a nonmodel system, but not to be surprised or resistant to lack of conservation. In particular, one should be open to taking an experimental approach that does not reference previous work in the model system, when it seems necessary, as this is the best way to discover taxonomically restricted biology.

Larvae

Rapid growth followed by molting is the only occupation of the larva in honey bees. The larva increases in size over the course of development, relative to size at hatching, by 900% in workers, 1700% in queens, and 2300% in drones (Winston, 1991). As shown in figure 3.3, this process only takes two to three weeks. Probably the only social behavior of larvae is that they produce a brood pheromone, which signals their collective hunger and guides the development of nurse bees (Le Conte et al., 1995, 2001).

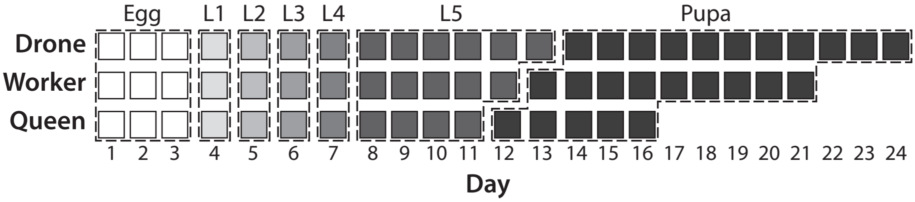


FIGURE 3.3. Variation in the length of development across honey bee castes. L1–5 represent the five larval instars (*redrawn from Cridge et al., 2017*).

In general, the mechanisms underlying growth in insects differ strongly from those in organisms with internal skeletons, like ourselves. This is because their exoskeleton, which is like a suit of armor, must be shed periodically for significant growth to occur. The study of molting is thus central to the understanding of growth in these systems. *Molting* refers to shedding the exoskeleton and reforming a larger one, with or without a change in morphological form. The period in between molts is a called an instar, and different orders of insects have variable numbers of instars and lengths of time they spend in them.

Honey bees go through six instars, independent of sex or caste. The first four instars occur with a period of one day each for all three castes (figure 3.4). During this period, the larva is in an open cell, is a simple grub-like creature, and is being monitored and fed by nurses (Jay, 1963b). The last two molts (fifth instar and pupa) vary considerably in length across the three castes. For the first two days of the fifth instar, the larva is fed by nurse bees and grows quickly. After this period, the cell is stocked with brood food and capped by the nurses. The larva then moves into an upright position and, with silk from the salivary glands, lines the inside of the cell with a thin cocoon (Jay, 1964). This takes about 36 hours and is sometimes called the spinning stage of the fifth instar. The larval cuticle is then shed in a process called apolysis, and a new pupal cuticle is formed. This is the prepupal stage (also called pharate pupa). Here the leg, wing, antennal, and gonadal imaginal discs of the larval epidermis evert and develop into their adult form. Imaginal discs are structures characteristic of holometabolous insects, set aside as epidermal packages during embryogenesis, which during the larval stages are kept in an undifferentiated state in pockets, well protected beneath the larval cuticle. During metamorphosis, these cells rapidly develop into adult structures (Belles, 2020). It is in the spinning stage that gut development is completed, allowing for defecation. The larva at this point is beginning to take adult form (Jay, 1963a).

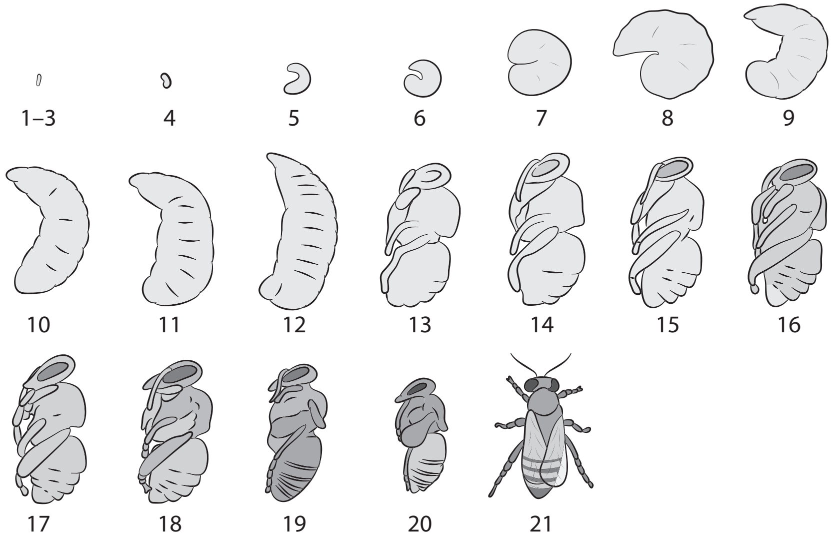


FIGURE 3.4. Morphology of worker larva through development (*redrawn from Wang et al., 2015*).

The next molt is to the pupal stage, the sixth and last instar, in which metamorphosis to adult form is completed. This involves a massive reorganization of the internal anatomy in a process that is still poorly understood in bees. Basically, the larval intestine, the dorsal aorta/heart tube, and the fat body are lysed, and the corresponding adult structures are rebuilt from nests of stem cells that remained quiescent in the larval stages. The nervous system is also restructured, marked by growth of the optic and antennal lobes of the brain and by the fusion of several ganglia of the ventral nerve cord. Externally, due to the unpigmented and soft pupal cuticle, the freshly molted pupa appears completely white, and the first change that becomes apparent is the gradual pigmentation of the eye's retina. As the eyes are gaining their full color, the pupal cuticle separates from the epidermis and a new adult cuticle is formed (Elias-Neto et al., 2009). The cuticle then takes on pigmentation and is sclerotized. This stage is also referred to as the pharate (or hidden) adult stage. Shortly before emerging from the brood cell, the bee (at this stage often called a teneral), sheds the pupal cuticle, which can still be seen as pieces adhering to the bee as she chews her way out of her cell. At this point, her cuticle is still soft, meaning she cannot yet fly or sting, her coloration is lighter than it ultimately will become, and she is quite hairy all over. She still has some development to complete before she begins her social work in the nest, but we cover these activities in chapter 12 since their study occurs in the context of division of labor.

Honey Bee Caste Determination

Caste determination is a process by which a totipotent diploid (female) egg develops into either a queen or a worker. It is a form of polyphenism, in which a common genome gives rise to radically different, usually discrete, phenotypes (Simpson et al., 2011, Hartfelder and Emlen, 2012). Polyphenism is not widespread in insects, but there are many well-studied examples. Aphids, which cycle through several morphs during the year; locusts, which can be stationary or gregarious; and many species of beetles, with alternative male morphologies associated with sexual selection, are examples having received experimental attention (Simpson et al., 2011, Hartfelder and Emlen, 2012). The social insects, particularly ants and termites with physical castes, are, along with aphids, the most complex known examples of polyphenism. The study of caste determination in bees has a special place, as this case of polyphenism has been the subject of many studies.

To understand caste determination in bees, we must explore three questions. First, what environmental triggers cause a larva to enter the queen or worker developmental pathway? Second, how is the external trigger translated into an internal pattern of endocrine signaling? Third, how do the hormones, and other molecular actors, orchestrate the gene expression differences associated with the production of caste-specific traits? Much progress has been made on each of these fronts, although relatively little has been accomplished with respect to linking them together.

Sugar Content Is a Key Trigger Leading to Queen Development

Differential feeding of the larva has long been known to be a major signal contributing to worker versus queen development (Asencot and Lensky, 1976, 1985, 1988). Nurse bees produce brood food from at least two glands, the hypopharyngeal glands and the mandibular glands (Winston, 1991). For the first three days of life, all eggs are fed with a combination of secretions from these two glands. Queen-destined eggs received a 1:1 ratio of the two glandular secretions, while workers receive a mixture strongly favoring the hypopharyngeal glands (Winston, 1991). The queen mixture is called royal jelly, while the worker mixture is called worker jelly. Queen-destined larvae are fed royal jelly throughout larval development. For workers, starting at the third instar they are fed a mixture of worker jelly and pollen (Haydak, 1970). Both castes are fed honey as well as the glandular secretions, and there is also a large difference in the quantity of food fed (Shuel and Dixon, 1959). The queen is always basically floating in copious quantities of food, while the workers are fed just enough. Recent work, in fact, suggests that quantity of food alone (independent of its nature) has a much larger role in caste determination than previously thought (Slater et al., 2020).

Given that two separate brood foods exist, the obvious question is, What differentiates royal and worker jelly? A key difference relates to sugar content. Early work showed that royal jelly contains more sugar than worker jelly, and experiments showed that worker-destined larvae that received supplemental sugar developed into queens (Asencot and Lensky, 1976, Kaftanoglu et al., 2011). In other words, if a larva is fed worker jelly that is supplemented with sugar only, this larva will develop into a queen. This is a key result, as it suggests that there is little in royal jelly relative to worker jelly, other than sugar, that is necessary for queen development. A popular hypothesis, based on these early studies, is that the high sugar content of royal jelly acts as a feeding stimulant that leads to more growth in queen-destined larvae (Goewie, 1978). This is difficult to test, of course, but given that worker-destined larvae do not have the opportunity to eat more than the little they are given suggests that this cannot be all that is going on.

Nonnutritive Triggers for Caste Development in Brood Food

Recent work has looked more closely at the idea that the trigger for queen or worker development is just nutritional in nature. The idea here is that worker and royal jelly may contain substances with the direct ability to alter gene expression in developing larvae. These caste-triggering substances fall into two categories. First, research has shown that worker jelly contains more microRNA than does royal jelly (Guo et al., 2013). Experimental supplementation of royal jelly with a microRNA found at high concentrations in worker jelly led to worker-biased development. A gene ontology (GO) analysis of the whole complement of microRNAs found in worker jelly suggested that they may be involved in brain development. Analysis of endogenously expressed microRNAs in developing workers and queens also supports the notion that microRNAs may be involved in caste effects related to brain development (Ashby et al., 2016).

The second substance in brood food with the capacity to directly control caste development is (E)-10-hydroxy-2-decenoic acid (10-HDA), which makes up 5% of royal jelly but only about 1.5% of worker jelly (Spannhoff et al., 2011, Wang et al., 2016). This fatty substance has been shown to be a histone deacetylase inhibitor. Acetylation of histones is one of the core epigenetic modifications controlling gene expression. Experimentally increasing levels of 10-HDA in brood food led to lower body size after pupation (Wang et al., 2014) and caused changes in the expression of the histone deacetylase 3 gene and DNA methyltransferase 3. This work is preliminary, however, and what role 10-HDA plays in caste determination is still unclear.

Are the Triggers Simple or Complex?

For many cases of insect polyphenism, an environmental trigger (crowding or photoperiod, for example) leads to a pattern of development such that once the trigger is received, development of the alternative morphologies goes to completion. There are no intermediate forms, just a branching pattern of discrete developmental pathways. In contrast to this simple notion of a trigger, it could be the case that the trigger of caste differentiation in honey bees is a continuous process with feedback between the nurses and the larva. In other words, there is no simple one-off signal, but rather a process of guided development (Linksvayer et al., 2009a, 2011).

This brings us to a core difficulty with understanding caste differentiation. The many traits that differ between workers and queens could have as many independent triggers. It could be the case that there is an environmental trigger, for example, for a barbed stinger and another for a pollen basket. Each trigger could be connected to a discrete hormonal and gene regulatory mechanism. Of course, an intermediate situation could also exist such that there are a handful of triggers, each of which controls a separate signaling pathway that controls some fraction of the caste-specific traits.

The simple prediction that distinguishes the simple switch and the complex pattern of one or more switches is the presence of intercastes (individuals with both queen and worker traits). A simple switch does not allow for intercastes while a complex pattern of switches does. Intercastes have long been known in honey bees (they are produced when worker-destined larvae up to three days old are used to produce queens), and thus it has always been thought that the trigger is not simple. Recent work, however, has shown that intercastes are much more variable than previously thought. Linksvayer et al. (2011) showed that when bees are reared in vitro on a diet containing royal jelly and sugar, the entire gamut of variation between worker and queen is observed. There is a nice bell-shaped curve with intercaste forms in the middle and queen and worker states at the extremes. This suggests that intercaste bees may be the default developmental program for honey bees and the behavior of the nurses selects for the most extreme phenotypes (Leimar et al., 2012). In keeping with this, queen traits can be induced in worker-destined larvae right up to the end of the fourth instar (before capping) by switching to feeding of royal jelly. These individuals are intercastes that are more worker-like than queen-like (Dedej et al., 1998), but this shows that caste differentiation is ongoing right up until the capping of the brood cell.

Endocrine Pathways Involved in Caste Differentiation

A handful of endocrine pathways have been shown to be central players in the regulation of growth and molting in model systems (Mirth et al., 2005, Layalle et al., 2008, Hatem et al., 2015). Specifically, insulin and insulin-like signaling (IIS) and the target of rapamycin (TOR) pathways are thought to control growth and nutrient signaling, while interactions between juvenile hormones (JH) and ecdysteroids control molting. Work in honey bees has focused on identifying how worker- and queen-destined larvae differ in the activity of these pathways. Early work highlighted the strong and clear differences in JH and ecdysteroid concentration through development in workers and queens, while recent work has explored the more subtle and difficult-to-characterize behavior of the IIS and TOR pathways (Hartfelder et al., 2015). Although IIS and TOR are probably upstream from JH and ecdysteroid, we cover them in the order in which they were experimentally elucidated.

JUVENILE HORMONE AND ECDYSTEROID

Early studies showed strong variation in the timing and level of JH and ecdysteroid titer in worker- versus queen-destined larvae (Dietz et al., 1979, Rembold et al., 1974, 1992, Ulrich and Rembold, 1983, Rembold, 1987). In particular, JH rates of synthesis and hemolymph levels are considerably higher in fourth and fifth instar queen-destined larvae than in worker-destined larvae and also show a small peak in queens during the cocoon spinning period (Rachinsky and Hartfelder, 1990, Rachinsky et al., 1990). Key experiments involved treatment of worker-destined larvae with JH, which under some conditions triggers queen development (Wirtz and Beetsma, 1972, Asencot and Lensky, 1976, 1984, Dietz et al., 1979, Copijn et al., 1979). JH signaling is thus a central player in caste differentiation.

High JH titer has also been shown to directly prevent the massive apoptosis of ovarioles that occurs in worker development, shown in figure 3.5 (Capella and Hartfelder, 1998, Hartfelder et al., 2018). Further, JH treatment of RNAi knockdown bees for the TOR and insulin pathways (discussed next) rescues the effect of knockdown, suggesting JH is downstream from these pathways (Mutti et al., 2011). The long-awaited discovery of the receptor for JH, Methoprene-tolerant (*Met*), and its associated transcription factor (TF), *Kr-h1*, will hopefully lead to a dramatic increase in our understanding of how JH (alone and in conjunction with ecdysteroids) controls gene expression during caste development (Charles et al., 2011, Jindra et al., 2015).

JH and ecdysteroids work together in a complex and changing manner to control caste differentiation in bees, but only bits and pieces of this relationship are currently understood. On a coarse-grained level, ecdysteroid levels in the hemolymph are higher in queen-destined larvae than in worker-destined larvae at the last larval instar (Rachinsky et al., 1990), and the two castes also differ strongly in ecdysteroid titers during the pupal phase (Pinto et al., 2002). With respect to the ecdysteroid receptor (EcR), it has been shown to be alternatively spliced with one variant expressed during embryogenesis and the other during larval development, a common pattern across the insects (Mello et al., 2014). The transcript expressed during larval development, *Ecr-A*, shows a large increase in expression during the cocoon spinning phases, both in workers and queens, but it is higher in workers. It then falls and rises during prepupal and pupal development with slight differences between castes.

With respect to linking the two hormonal pathways, ecdysteroid release has been shown to be controlled by JH, at least in part (Hartfelder et al., 2015). This was shown by treating fifth instar worker larvae with synthetic JH III and showing an elevation of ecdysteroid synthesis and release from the prothoracic gland as a consequence (Rachinsky and Engels, 1995, Hartfelder and Engels, 1998). This relationship between JH and ecdysteroid seems to change pre- and postpupation, however, as application of a JH analog postpupation delayed and decreased the pupal ecdysteroid peak (Zufelato et al., 2000). Such transitions are common in insect development, as they are associated with transitions from one state to another in which a new regulatory circuit is necessary (Belles, 2020). Of course, this complexity makes working out the

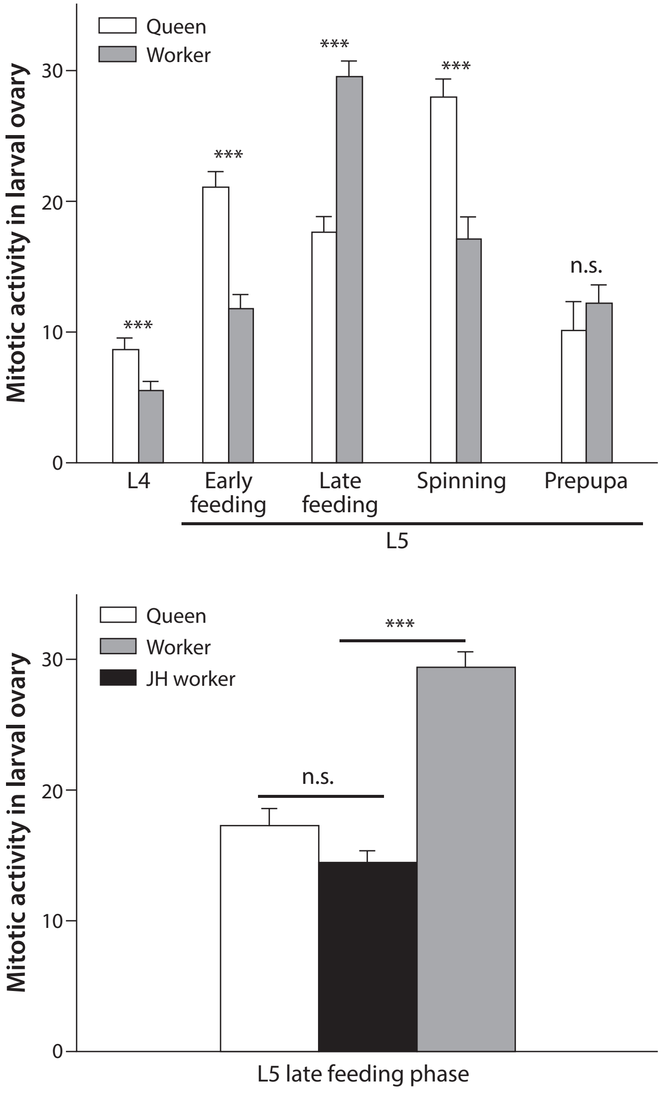


FIGURE 3.5. Mitotic activity in ovaries of worker- and queen-destined larvae. Top: Activity through development in unmanipulated larvae. Bottom: Worker-destined larvae have queen-like activity levels after JH application (*from Capella and Hartfelder, 1998*).

regulatory biology of this process difficult. Finally, caste-specific differences in ecdysteroid concentration have been shown to affect ovary development prior to the pupal phase and to be involved with the development of the cuticle and brain later in pupation (Barchuk et al., 2002, 2007).

TOR AND INSULIN SIGNALING

The TOR and IIS pathways are highly conserved pathways associated with nutrient sensing and growth across the Metazoa. The TOR gene, a kinase, is more highly expressed in queen- than in worker-destined larvae (Patel et al., 2007). Further, RNAi knockdown of TOR in larvae leads to the development of a worker-like phenotype (Mutti et al., 2011). Modulation of TOR function with a pharmacological inhibitor, rapamycin, also biases development toward workers. Similar results have been found for IIS signaling. Honey bees have two insulin-like peptides, and each is differentially expressed (in a complex manner) between queens and workers during larval development (Wheeler et al., 2006, de Azevedo and Hartfelder, 2008). RNAi knockdown of the insulin receptor leads to the production of workers even when larvae are fed a queen-destined diet (Mutti et al., 2011, Wolschin et al., 2011).

The research we have reviewed thus far suggests that TOR and IIS are involved in caste differentiation and are probably upstream from JH and ecdysteroid signaling. They are further likely involved in the setting of growth rate and the optimal size at molting between castes, given the role of these genes in model systems. But, to date, these studies should probably be thought of as preliminary. The reason for this is worth exploring to give the reader a sense of the difficulty of this branch of bee biology.

If we imagine knocking down the expression of key TOR or IIS components in a solitary bee species without polyphenism, we would expect to get strong variation in multiple adult phenotypes. The adults might be bigger or smaller, or the ovaries might be bigger or smaller. Of course, this variation would have nothing to do with polyphenism since it does not exist in this hypothetical solitary bee. We are merely toying with the core regulatory mechanisms controlling these traits. We must consider the work on IIS and TOR in honey bees in this light. Modulation of IIS or TOR function has been shown to cause a bigger or smaller size or variation in ovary development, and this is interpreted as being due to the key role these pathways play in the developmental programs underlying caste. However, IIS and TOR are so fundamental to all growth and nutrient signaling that disruption to them must have strong effects independent of anything having to do with caste. It is thus quite difficult to interpret the meaning of crude experiments knocking down total function of these core pathways. Until we have the ability to modulate in a dose-dependent manner the activity of different components of these pathways, and their interaction partners, it will be difficult to understand the role of IIS and TOR in caste differentiation. Given the rapidity with which CRISPR/Cas9 is being crafted for use in nonmodel systems, this day may not be too far off. Roth et al. (2019), for example, produced the first fully mutated bee (*dsx* knockout) using CRISPR/Cas9.

Epigenetic Effects on Caste Differentiation

Epigenetics is becoming a vast subject, which means we cannot give a satisfying overview here. We can give the reader the gist of the field, however, and quickly cover what work has been done in bees in the context of caste differentiation. In broad terms, epigenetics refers to heritable changes that do not involve changes to the DNA sequence (Goldberg et al., 2007). In practice, it usually refers to heritable patterns of gene regulation within cell types. Liver and kidney cells, for example, reproduce their own kind after mitosis, even though both cell types have the same genome. Cases of patterns of gene regulation being transmitted from parent to offspring have also been discovered, but they are not germane to our discussion.

There are many ways that information can be passed epigenetically, but most research has focused on two mechanisms. Methylation of cytosine bases has been shown to be widespread across the tree of life (Bird, 2002). Levels of methylation vary across the genome in a taxonomically variable manner, and the effect of methylation also depends on the taxonomic group. In mammals, for example, the genome seems to be broadly methylated with a bias toward transcription start sites (TSSs). Some evidence suggests that methylation may be involved in downregulation of expression in mammals (Jones and Takai, 2001). In insects, methylation is highly heterogeneous across the genome such that some genes are highly methylated and others are not. There is evidence suggesting that housekeeping genes in insects are those most likely to be methylated (Glastad et al., 2011). Furthermore, and different from mammals, methylation marks in insect genomes are generally not on the promoter region of a gene, but within the coding region, called gene body methylation (Yan et al., 2015).

Marks—technically speaking, posttranslational modifications (PTMs)—can also be added to the histones, the proteins used to package DNA in the nucleosomes (Lennartsson and Ekwall, 2009). Some common histone PTMs are acetylation, methylation, phosphorylation, and ubiquitination. PTMs influence the interaction between DNA and the histone, affecting the exposure of DNA to transcriptional machinery. It is currently thought that the effects of methylation of DNA and histone PTM can work together or separately to control gene expression. The term *chromatin remodeling* is used to refer to the processes of packing and unpacking DNA bound up in histones.

We reviewed earlier that 10-HDA, which is a major component of royal jelly, has a histone deacetylase inhibitor activity in mammalian cells (Spannhoff et al., 2011), indicating a possible effect for chromatin remodeling in honey bee caste determination. Honey bees also have three DNA methyltransferases, the enzymes that methylate DNA, encoded in their genome (Wang et al., 2006). The RNAi-mediated knockdown of DNA methyltransferase 3 function in larvae showed a strong effect in biasing their development to a queen phenotype, especially for the ovaries, as shown in figure 3.6 (Kucharski et al., 2008). Adult workers and queens have also been shown to have different patterns of methylation across their genomes in brain tissue (Lyko et al., 2010).

Two recent studies have expanded our understanding of chromatin remodeling in caste determination. Dickman et al. (2013) conducted a broad search for PTMs on three histone proteins (H3.1, H3.3, and H4) in 96-hour-old worker larvae and in queen ovaries. This was done via protein extraction followed by mass spectrometry. They verified that bees make use of extensive histone modifications, identifying 23 different PTMs. H3K27 and H3K36 in particular were sites of combinatorial marks (multiple PTMs) in queen ovaries. A follow-up study by Wojciechowski et al. (2018) used a combination of ChIP and RNA seq to explore the global pattern of histone PTMs on developing worker and queen larvae at 96 hours. They focused on three PTMs known from other systems to play important roles in gene regulation (H3K4me3, H3K27ac, and H3K36me3). They first validated that these PTMs play conserved roles in honey bees. H3K4me3 and H3K27ac are enriched around TSSs, while H3K36me3 is downstream from TSSs and probably marks the end of the gene. All three PTMs showed caste-specific patterns across the genome. H3K4me3 and H3K36me3 correlated with differential expression (based on RNA seq) between castes, suggesting a role for the regulation of these genes. H3K27ac did not correlate with overall gene expression but showed a stronger spatial bias between castes. Queen-specific sites were in

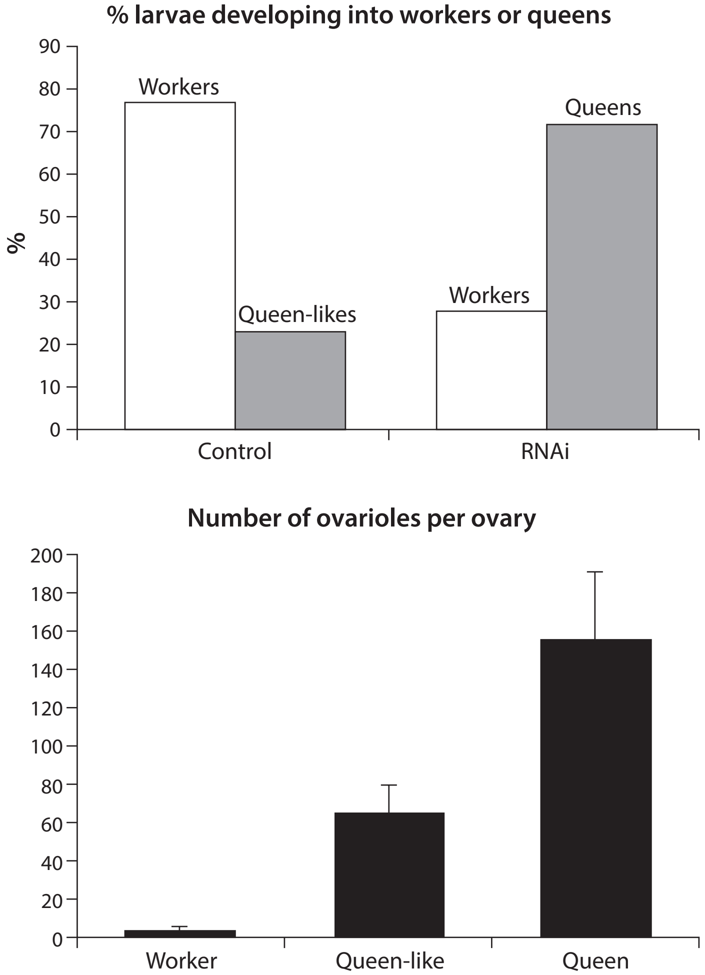


FIGURE 3.6. Effect of silencing Dnmt3 on caste determination. Top: Percentage of bees developing into worker or queen forms. Bottom: Ovarioles per ovary for different larval outcomes (*from Kucharski et al., 2008*).

exons and close to TSSs, while worker sites were more variable but often associated with introns. Those marks associated with workers did correlate with caste-specific expression, and the authors interpret this as suggestive that they are enhancers of gene expression. An analysis of TF binding sites associated with H3K27ac further supports this notion.

Undoubtedly, we have only seen the tip of the iceberg with respect to epigenetic effects on this and most other processes. Epigenetic control of caste determination should therefore be a rich topic for future study, and what is currently known in bees indicates that the honey bee could become a model insect for this topic, given that *Drosophila* does not have a full complement of the epigenetic machinery.

4

Anatomy and Physiology

This book provides an up-to-date, comprehensive treatment of honey bee biology. This involves coverage of results long known to science and topical exploration of new ideas. Needless to say, honey bee anatomy has not changed recently and there are many wonderfully detailed references. Snodgrass (1956) is the standard reference. It has detailed plates of every anatomical and morphological structure. The reader interested in high-resolution illustrations and detailed discussions of bee anatomy and morphology is referred to that book. In this chapter, we present an overview of anatomy and morphology appropriate for biologists in general, but not sufficiently detailed for systematists or those specializing in anatomy or physiology.

With respect to physiology, we focus on core principles and how they are realized in honey bees. Much of this information is not specific to honey bees, but it is critical to present it because insect physiology is radically different from vertebrate physiology and only those with specialized training in entomology have a working understanding of it. This creates problems for the biologist who switches from a noninsect taxon to honey bees, a situation that has become common in recent years. We also cover nutrition and the microbiome, a rapidly growing field.

Bee Morphology and Anatomy

Due to the profound polyphenism present in honey bees, they have three basic forms to describe rather than the two typical for most species. Males and the two female morphs (workers and queens) are each quite distinct in morphology and anatomy (plates 4–6). Our discussion emphasizes the worker bee and

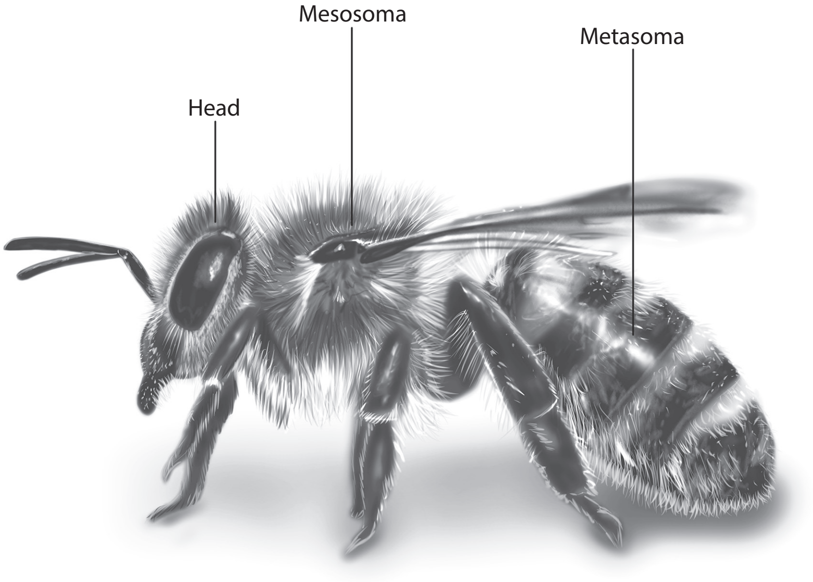


FIGURE 4.1. The three body regions of the honey bee (*original artwork by Maather Basfar*).

explains how queens and males differ from it. We organize our presentation with reference to the three major body sections: the head, the mesosoma (thorax), and the metasoma (abdomen), as shown in figure 4.1.

The Head

The worker bee's head and mouthparts are shown in figure 4.2 (Snodgrass, 1956). The head is home to most of the sensory structures, the mouthparts, many important glands, and the brain. We cover the brain when we discuss neurobiology in chapter 6.

ANTENNA

The insect antenna is a multipurpose sensory organ involved in many processes. It is the primary organ of olfaction, of course, but it is also involved in sound reception, the detection of flight speed, and the detection of humidity

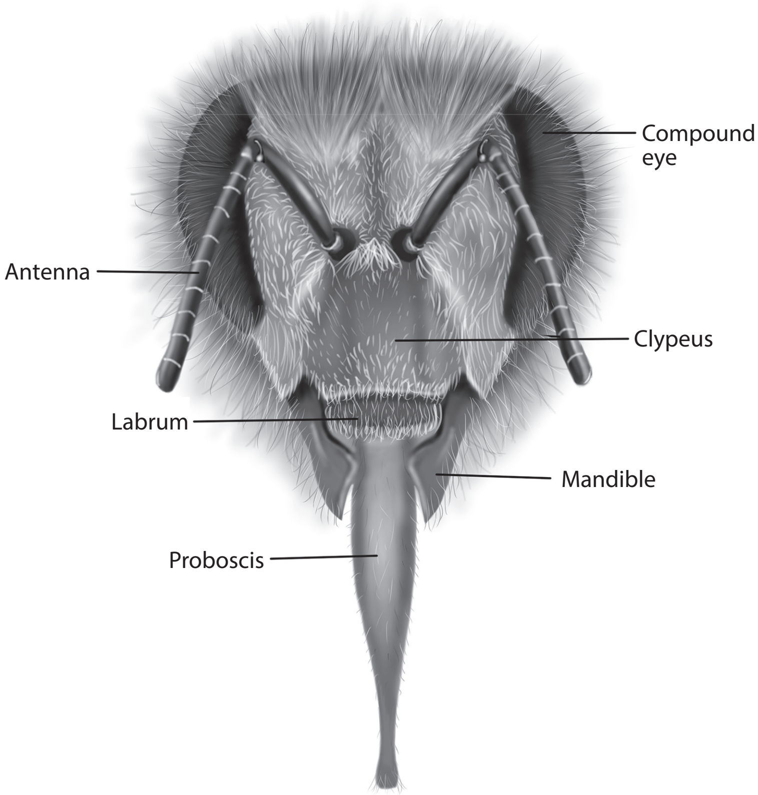


FIGURE 4.2. The head and associated structures (*redrawn from Snodgrass, 1956*).

(Klowden, 2013). Its basic structure is shown in figure 4.3. The Johnston's organ, which is in the pedicel, the second segment of the antenna, is a complex chordotonal organ found in all insects. In bees, this structure is involved in the reception of sound and is likely involved in decoding the dance language (Tautz, 2008).

EYES

The compound eyes are the primary visual organs. They differ from our own camera lens eyes by having a multitude of small lenses, each of which independently collects visual information that is then integrated in the brain into

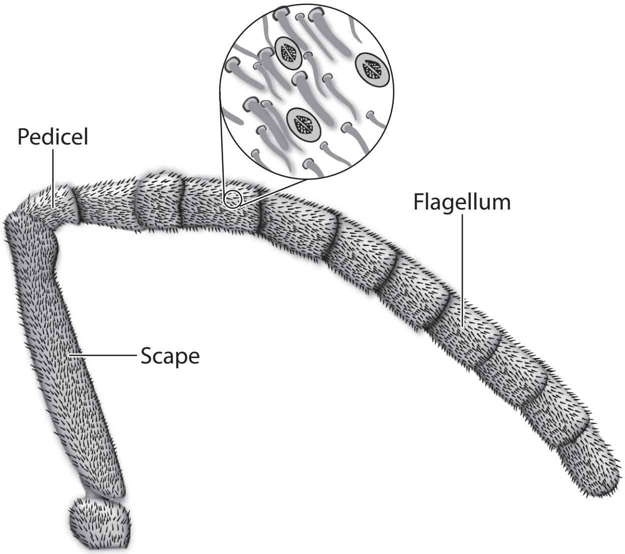


FIGURE 4.3. The worker antenna (*redrawn from Tsujiuchi et al., 2007*).

one image (Land, 1997). See chapter 6 for a detailed discussion of form and function relating to vision. The ocelli are three simple eyes on the top of the bee's head (figure 4.4). These eyes do not form an image, but are sensitive photometers thought to be critical for regulating orientation during flight (Klowden, 2013).

MOUTHPARTS AND ASSOCIATED GLANDS

The mouthparts of the honey bee are shown in figure 4.5. The proboscis, formed from components of the maxillae and labium (often called the labiomaxillary complex), is used to drink fluids. It is a complex organ that folds up and out of the way when not in use (Danforth et al., 2019). When in use, the outer parts form an airtight straw through which the glossa, or tongue, is inserted. Powerful muscles connected to the base of the glossa (called the cibarial pump) create suction (Snodgrass, 1956). The proboscis is also used within the nest in the transport of pheromones and in trophallaxis (food sharing). The mandibles are used to manipulate objects, shape wax, smooth down rough surfaces, and hold liquid during trophallaxis. They are also used in fights.

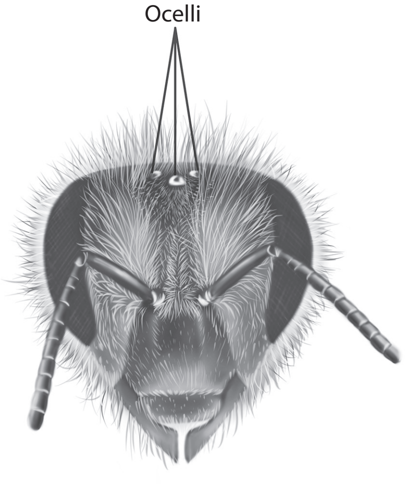


FIGURE 4.4. The worker ocelli (*original artwork by Maather Basfar*).

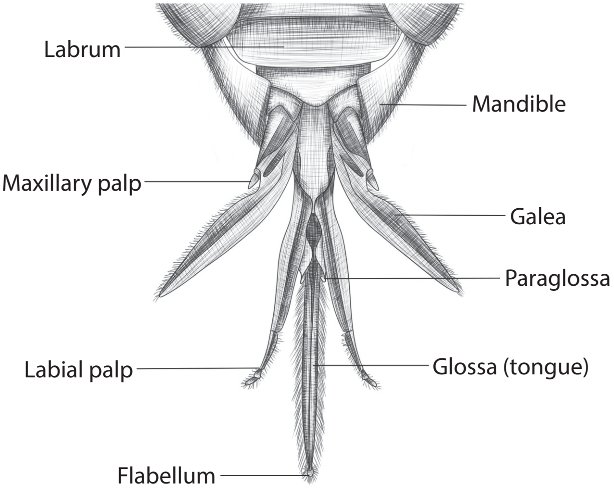

Labrum
Mandible
Maxillary palp
Galea
Paraglossa
Labial palp
Glossa (tongue)
Flabellum

FIGURE 4.5. The worker mouthparts (*redrawn from Snodgrass, 1956*).

Unlike stingless bees, who use their powerful mandibles for defense against vertebrates, the honey bee's mandibles are not strong enough to break human skin or even to exert enough pressure for a painful pinch.

There are three large glands associated with the mouthparts (figure 4.6). Two of these, the hypopharyngeal glands and the mandibular glands, have

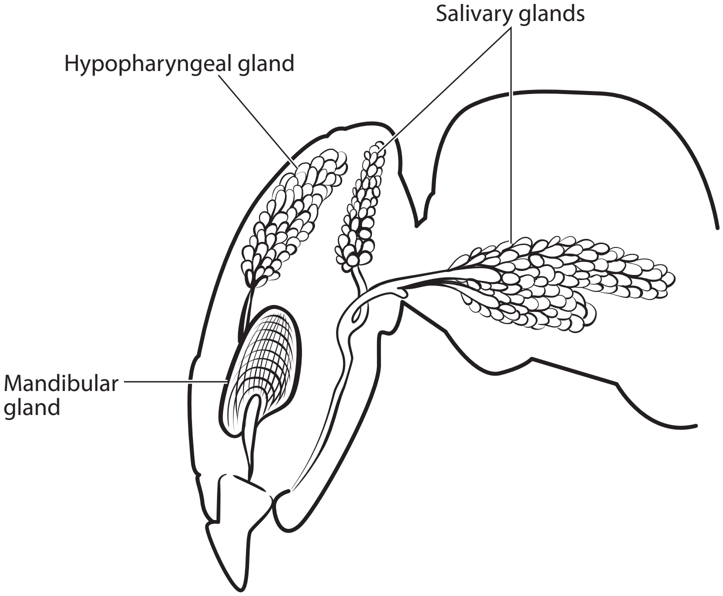


FIGURE 4.6. The glands associated with the worker's mouthparts (*redrawn from Snodgrass, 1956*).

received considerable attention from biologists. The hypopharyngeal glands are the largest glands in the bee's head, and perhaps in the whole body (Huang and Otis, 1989). These glands contribute to brood food in nurse bees and nectar processing enzymes and defensive secretions in foragers (Fluri et al., 1982, Crailsheim, 1991, Vannette et al., 2015). The mandibular glands articulate with the mandibles, giving them their name. These glands also contribute to brood food in nurses and defensive secretions in foragers (Vannette et al., 2015), but they have received the most attention for their role in the production of queen pheromone. This complex pheromone blend is important for signaling to the hive that a viable queen is present (Slessor et al., 2005).

The final glands are the salivary glands, which in honey bees are broken into two distinct sections—one in the head and the other in the abdomen. The two sections, cephalic and thoracic, have different functions (Simpson, 1960, Winston, 1991). The cephalic section produces a milky secretion and the thoracic section a watery one. Recently, Martin et al. (2018) showed that the cephalic portion of the salivary gland secretes the hydrocarbons used in nestmate recognition.

The Mesosoma

The early ancestors of insects were highly segmented. For the head, the ancestral sections are not visible, but for the thorax and abdomen the individual segments are generally distinct. The thorax has three segments, each with an associated pair of legs. In bees and related aculeate wasps, the first abdominal segment, called the propodeum, has become associated with the three thoracic segments that form the mesosoma. The remaining segments of the abdomen (Ab2–Ab8) form the metasoma. In general, the mesosoma is dominated by the demands of locomotion whereas the metasoma is associated with digestion and reproduction.

LEGS

The honey bee, like all insects, has three pairs of legs (figure 4.7). The coxa connects to the thorax, and the rest of the leg is made up of the trochanter, the femur, the tibia, and the five-part segmented tarsus. Each pair of legs is associated with one thoracic segment. All legs have some degree of specialization. The first pair of legs, for example, contains a comb that is used to clean the antenna. The midlegs have a hook that helps the bee move wax scales from the abdomen to the head area so that it can be molded into comb. The hind legs contain the pollen basket, or corbicula, which is used to transport pollen and propolis. This structure creates a sizable indentation on the outer side of the leg with a large bristle running through it that the bee uses for collecting and storing pollen. All three pairs of legs work together in complex patterns to collect pollen grains from the bee's hairy body and pack them into the pollen basket. A similarly complex set of behaviors, this time including the mandibles, is used to collect and pack propolis. The tibia contains an important chordotonal organ called the subgenual organ. This organ is the means by which bees perceive substrate vibrations (Kilpinen and Storm, 1997, Storm and Kilpinen, 1998). The tarsus includes the claw and pad (arolium) at the end of each leg. This structure is important for grasping objects and climbing smooth surfaces. It is also associated with the tarsal gland.

WINGS

The wings of insects are outgrowths of the cuticle, not modified appendages. Bees have two pairs of wings that connect with a series of hooks called hamuli (figure 4.8). As for many insects, the wingbeat frequency is much faster than

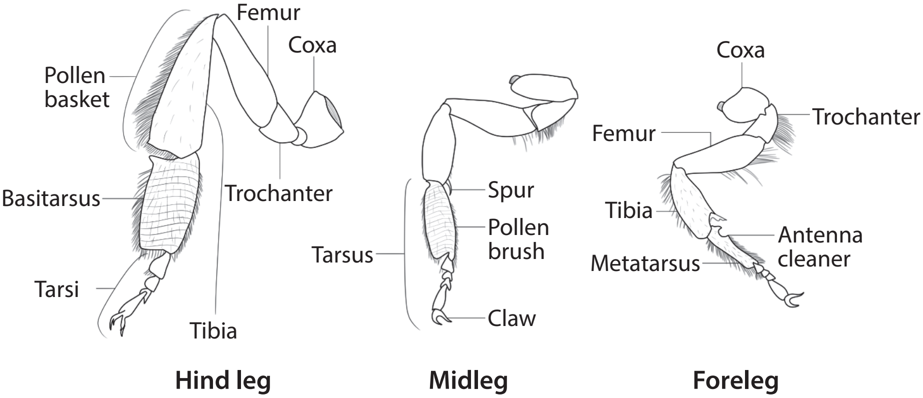

Femur
Coxa
Pollen basket
Basitarsus
Trochanter
Tarsi
Tibia
Spur
Pollen brush
Tarsus
Claw
Coxa
Trochanter
Femur
Tibia
Antenna cleaner
Metatarsus
Hind leg
Midleg
Foreleg

FIGURE 4.7. The worker bee's legs (*original artwork by Maather Basfar*).

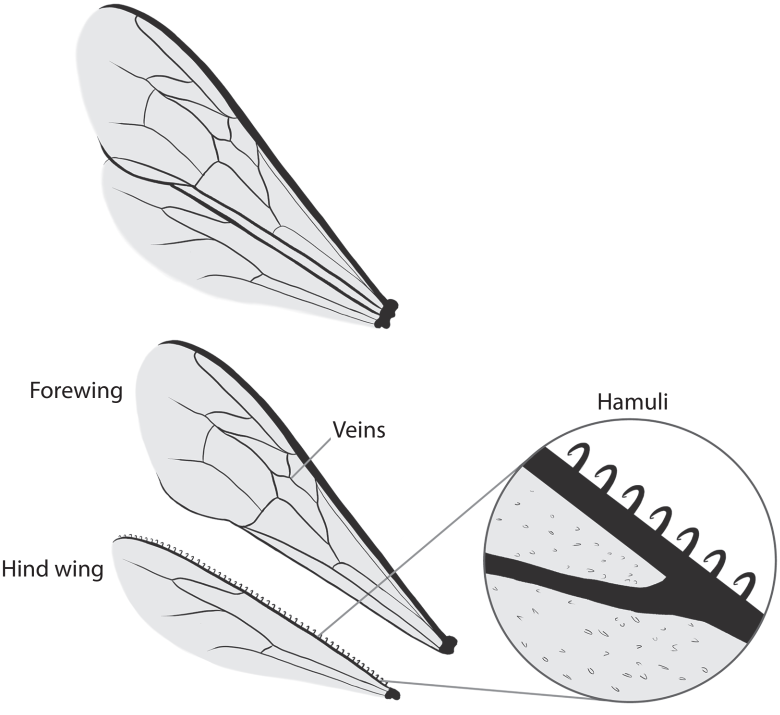

Forewing
Veins
Hamuli
Hind wing

FIGURE 4.8. Wings of the worker bee with hamuli (*redrawn from Snodgrass, 1956*).

the maximum rate that neurons can fire, meaning that one nerve impulse is associated with many beats of the wings. This is accomplished by taking advantage of the mechanical energy stored in the rigid thoracic cuticle when it is physically deformed due to contractions from the powerful thoracic muscles. Essentially, the muscle plucks the thorax and then vibrates to move the wings. This is often called indirect flight.

With respect to internal anatomy of the thorax, the flight muscles are huge and fill nearly the whole thorax. They exhibit a sharply striated pattern in young bees but become heavily worn and take on a shredded appearance in older foragers. This is indicative of the amount of work bees do over the course of their lifetime. In fact, it is often argued that honey bees may endure the harshest metabolic and temperature stresses known in the animal kingdom as they busily forage (Elekonich, 2009, Margotta et al., 2014). Early work showed that the temperature problems are handled with an ingenious system of evaporative cooling, whereby heat from the thorax is quickly transferred to the head where a droplet of nectar is excreted to cause evaporative cooling (Heinrich, 1980a, 1980b). Recent work has focused on the molecular mechanisms whereby the oxidative stress caused by the work of the flight muscles is handled. This work has shown a connection between flight activity and senescence (Margotta et al., 2014), but has shed little light on how bees mitigate these effects.

The Metasoma

The bee abdomen is the simplest body region from an external morphological perspective, but internally it is home to most of the physiological systems. We cover the digestive system, the fat body, the circulatory system, the stinger and its associated glands, and some other important glands.

DIGESTIVE SYSTEM

Figure 4.9 shows the digestive system of the honey bee. It is rather straightforward with one exception—the crop. The crop is a sack separated from the stomach where food can be stored free from digestive fluid contamination. Foragers store the water and nectar they collect in their crops. They transfer crop contents to nectar processors in the nest, who also store these substances in their crops. It is thus a carrying case. When empty the crop is quite small, but when full it can fill nearly the whole abdomen. Otherwise, bees have a

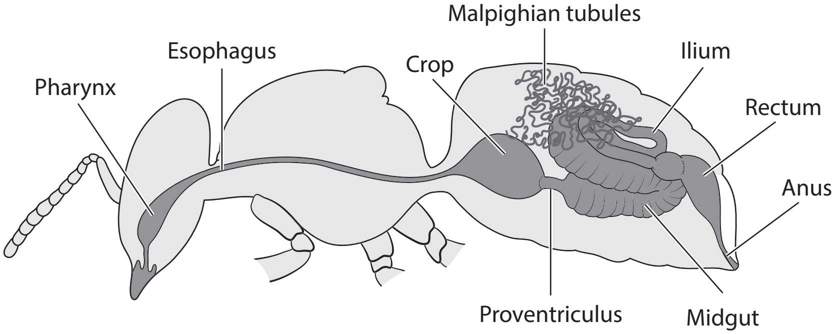


FIGURE 4.9. Digestive system of the worker bee (*redrawn from Snodgrass, 1956*).

foregut (including the esophagus and crop), midgut (where absorption takes place), and hindgut (ileum and rectum). Both the foregut and hindgut are lined with cuticle called the peritrophic matrix. These structures perform functions largely conserved across the Metazoa. The Malpighian tubules function in the elimination of waste from the hemolymph and are analogous to the vertebrate kidneys.

THE FAT BODY

The fat body is a thick layer of white or cream-colored cells that coats the interior of the abdomen. The fat body has many functions and is widely studied in the honey bee (Gullan and Cranston, 2005, Arrese and Soulages, 2010). As its name suggests, it stores fats, but it also stores glycogen and proteins. This is a key difference between vertebrates and insects, as we store food reserves as fat and a little carbohydrate, but we do not store protein. Insects, in contrast, store large amounts of fats, proteins, and carbohydrates. The fat body also produces vitellogenin, the yolk protein that has many novel social functions in honey bees (Amdam et al., 2003a, Guidugli et al., 2005). In fact, the fat body is the place of most protein production, with lipid transport proteins, heat shock proteins, and various storage proteins being produced there. Nutrient sensing, which is key to growth and maintenance in all organisms, also occurs mainly in the fat body (Ament et al., 2008, 2011). The fat body is also an important component of the insect immune system and plays a role in the detoxification of toxic compounds (Evans et al., 2006, Lehrer and Ganz, 1999, Berenbaum and Johnson, 2015).

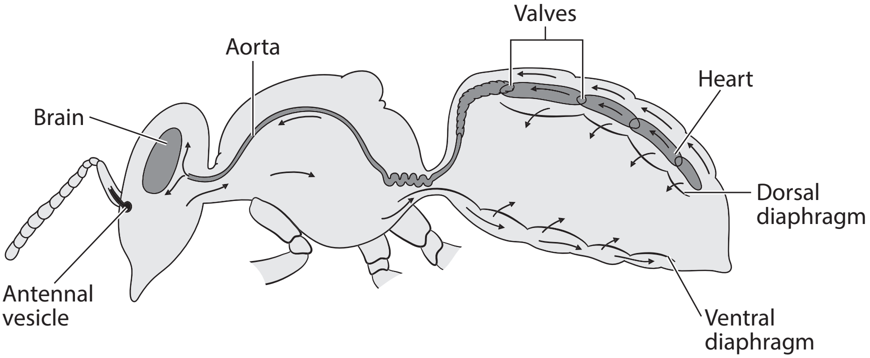


FIGURE 4.10. Worker circulatory system (*redrawn from Snodgrass, 1956*).

THE HEART AND HEMOLYMPH

Bees have an open circulatory system, which contrasts sharply with the closed circulatory systems of vertebrates (figure 4.10). Essentially, the bee's blood, called hemolymph, is not enclosed in tubes under pressure. Rather, it sloshes around in the body cavity. Mixing of the blood, to homogenize the concentration of various solutes, is achieved by blood vessels, but these are quite rudimentary. The bee's heart, for example, is an open tube connected to the dorsal surface of the bee's abdomen and extending to the head. It moves blood in one direction, from back to front, with the aid of muscular contractions and one-way valves. The blood of insects is also quite different from that of vertebrates. Chief among the differences is that bee blood does not transport oxygen. Gas exchange in insects is achieved with a tracheal system, covered later in this chapter. As is true for our blood, insect blood is involved in transporting nutrients, in mounting immune responses, and in the transport of endocrine signals.

THE STINGER

The honey bee stinger is perhaps its most famous anatomical trait. Figure 4.11 shows the stinging apparatus. In workers, a barbed stinger saws into the flesh of a vertebrate attacker and sticks, taking with it the sting gland and the necessary machinery to ensure that the sting gland continues to pump venom into the attacker (plate 7). It is interesting to note that the stinger is not ripped out when bees sting insect attackers, such as other honey bees. The barbs are thus an adaptation for ensuring that large vertebrate predators get the maximum dosage of venom from each sting. It is therefore a common misconception that

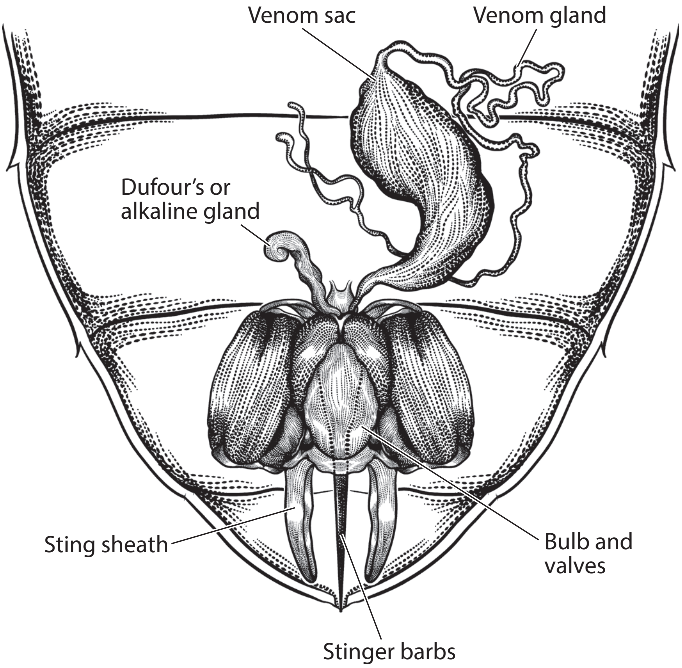


FIGURE 4.11. The sting apparatus and associated glands (*redrawn from Snodgrass, 1956*).

wasp stings are worse than bee stings because they can sting repeatedly. The honey bee stings once (when stinging vertebrates), but she gives her maximum dosage of venom, while wasps only deliver a small dose of venom per sting. Of course, the composition of the venom matters greatly for how painful a sting is, but in general honey bees have powerful stings.

In terms of gross anatomy, the stinger is connected to the venom sac, which connects to the venom gland (Cassier et al., 1994). The Koschevnikov gland (not shown), which produces alarm pheromone, is also located close to the stinger (Schmidt et al., 1997, Bortolotti and Costa, 2014). Only females have stingers, as they are modified ovipositors. Queen bees have quite distinct stinging biology, which is covered at the end of the chapter.

The composition of bee venom has been known for some time (Benton et al., 1963, Habermann, 1972, Gauldie et al., 1976, Hider, 1988, Komi et al., 2018). In a nutshell, bee venom is a complex cocktail of compounds designed to elicit pain, swelling, and itching. Table 4.1 shows the major components (Hider, 1988). Melittin is a small peptide that disrupts cell membranes (Kreil, 1973, Dawson et al., 1978). Nearly half of venom is composed of melittin. Phospholi-

Table 4.1. Contents of honey bee venom

Type of compound	Components	Percentage of venom composition
Proteins	Hyaluronidase	1%–3%
	Phospholipase A2	10%–12%
Peptides	Melittin	50%
	Secapin	0.5%–2.0%
	MCD	1%–2%
	Tertiapin	0.1%
	Apamin	1%–3%
	Procamine	1%–2%
Amines	Histamine	0.5%–2.0%
	Dopamine	0.2%–1.0%
	Noradrenaline	0.1%–0.5%
	Alpha-aminobutyric acid	0.5%
Sugars	Glucose	2%
	Fructose	0%
Phospholipids		5%
Alpha-amino acids		1%
Volatile compounds (pheromones)		4%–8%

Source: Hider, 1988.

pase A, an enzyme, has a similar function and works synergistically with melittin to shred cell membranes (Shipolini et al., 1971, King and Spangfort, 2000). Hyaluronidase breaks down the glue holding the skin together to allow the venom to penetrate further. Apamin, secapin, and tertiapin are neurotoxins, while MCD releases histamine, which may cause swelling (Vincent et al., 1975, Hugues et al., 1982, Taylor et al., 1984, Rehm et al., 1988, Drici et al., 2000, Komi et al., 2018).

OTHER ABDOMINAL GLANDS

There are three additional glands located in the abdomen if we limit ourselves to queens and workers. These are the wax glands, the Nasonov gland, and the Dufour's gland (figure 4.12). We only briefly introduce them here, as they are the subject of considerable discussion in later chapters. The wax glands secrete wax scales used to build the nest. These paired glands are located on the ventral surface of the abdomen. Wax glands are closely associated with the fat body

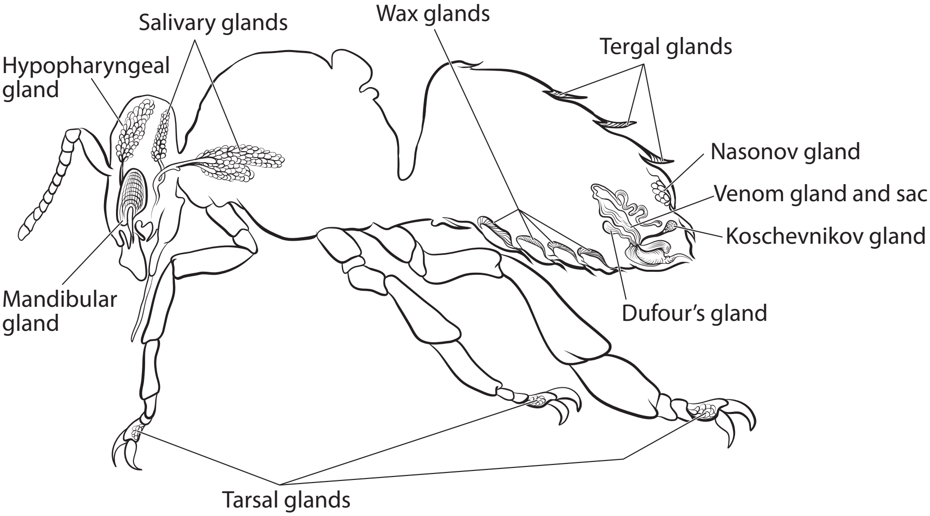

Salivary glands
Wax glands
Tergal glands
Hypopharyngeal gland
Nasonov gland
Venom gland and sac
Koschevnikov gland
Mandibular gland
Dufour's gland
Tarsal glands

FIGURE 4.12. Honey bee glands (*redrawn from Snodgrass, 1956*).

(Cassier and Lensky, 1995). The Nasonov gland produces a pheromone used as a homing beacon (Cassier and Lensky, 1994). It is located on the dorsal side of the abdomen just before the last tergite. The Dufour's gland is associated with reproductive signaling in queens and workers (covered in chapter 14), a highly derived function with respect to its ancestral function in solitary bees, which is to produce the brood cell lining (Hefetz, 1987, Katzav-Gozansky et al., 1997, 2001, Nino et al., 2013a, Danforth et al., 2019).

Insect Physiological Systems

For some physiological systems, we have found it convenient to give a synopsis of their function as we describe the associated anatomical structures. This is particularly appropriate for those systems limited to a single body segment. Here we cover the remaining physiological systems, with the exception of the nervous system.

The Skeleton

Unlike vertebrates, which have an internal skeleton, bees have an exoskeleton, or integument (Gullan and Cranston, 2005). Common to all skeletons, it provides support for the weight of the body and attachment points for muscles. Unique to the exoskeleton, it must be shed for the animal to grow. The cuticle also functions as a barrier between the organism and the environment, which is important for relegating water loss and protection against parasites and pathogens.

The exoskeleton is a complex structure. It is made up of several layers: the epicuticle, the exocuticle, the endocuticle, and the epidermis (Vincent and Wegst, 2004). The thin epicuticle is a rather complicated structure with multiple layers. The outer cement layer is the primary barrier between the insect and the environment, while the interior is waxy and serves to prevent desiccation. Coloration and chemical signaling also take place here. Both the exocuticle and the endocuticle are composed of chitin plus a diversity of proteins. They make up the bulk of the integument, which is a sizable fraction of the insect biomass. The exocuticle is the hardest part of the exoskeleton, having undergone a process of sclerotization. It is what is shed during molting. The endocuticle provides most of the strength to the skeleton, and because it is not sclerotized, provides for flexibility. It is reabsorbed and recycled during the molting process. In regions of the body requiring more flexibility, the cuticle contains a considerable amount of resilin (a rubbery protein). The epidermis is living tissue that secretes the other layers. Integrated into the exoskeleton are a great many glandular structures with associated canals, and sensory structures.

Circulatory System

Insects do not use a carrier molecule, such as our hemoglobin, to transport oxygen. Gas exchange occurs directly from cells to the air (Gullan and Cranston, 2005). Fresh air is circulated throughout the bee's body through its tracheal system (figure 4.13). The tracheal system starts with large trunk tubes that connect to the outside via specialized openings called spiracles, which can be opened or closed to regulate gas composition. As the tracheal tubes branch into the body cavity, they become increasingly fine until they finally reach every cell in the body. The tracheal system is part of the cuticle and must be shed during molting. For holometabolous insects, like the honey bee, this only occurs during the juvenile stages.

Endocrine System

The endocrine system is involved in the coordination of internal activity, at both local and global scales. Every aspect of development, physiology, and behavior is at least partially controlled by the endocrine system. The endocrine system works via the sending and receiving of chemical signals, hormones,

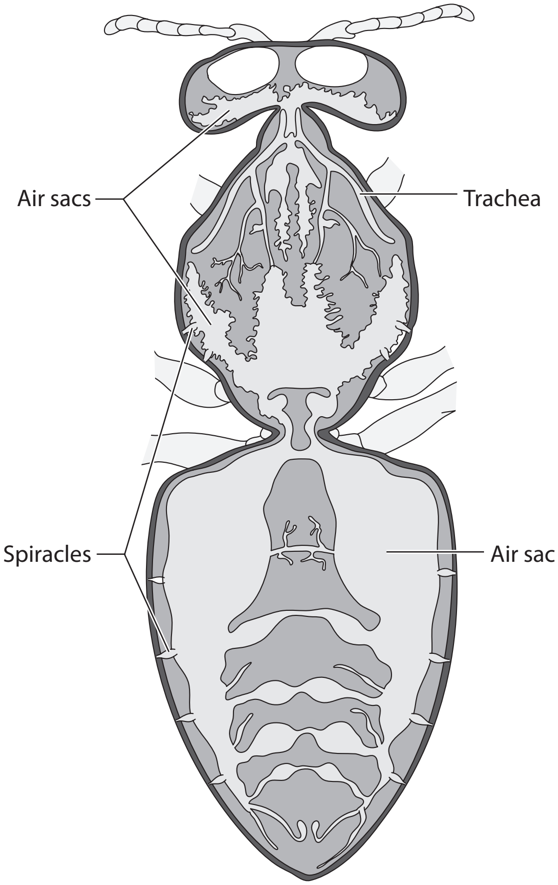


FIGURE 4.13. Worker tracheal system (*redrawn from Snodgrass, 1956*).

and other such molecules. In honey bees, the endocrine system is key to understanding division of labor, caste-specific development, and a host of other well-studied phenomena.

The key endocrine centers are the neurosecretory cells in the brain, which coordinate many functions centered on growth and reproduction; the corpora cardiaca, also in the brain and near the heart, which is involved in molting; the prothoracic glands in the first segment of the thorax, which are also involved in molting; and the corpora allata in the abdomen, which secretes juvenile hormone, central for growth, molting, reproduction, and social behavior (Klowden, 2013). The reproductive tissues are also sites of endocrine signaling.

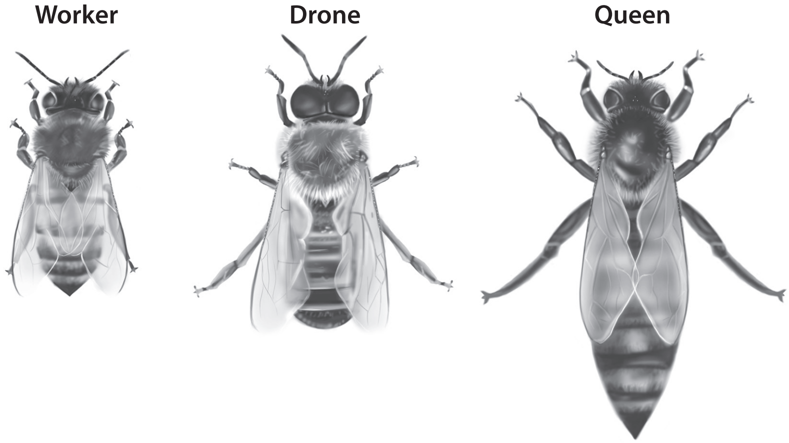


FIGURE 4.14. Queen, drone, and worker body forms (*original artwork by Maather Basfar*).

Contrasting Queens and Workers

The clearest external morphological difference between queens and workers is their size. Queens are larger than workers, and their body has a characteristically different shape, which is most pronounced when they are laying eggs (figure 4.14). Like drones, queens lack pollen baskets and many other structures that workers use in various social activities. The queen's proboscis, for example, is smaller than that of the worker, consistent with the fact that she never forages. Queens also have smaller eyes (fewer facets) and antennae with fewer sensory plates. Queen sensory systems are thus reduced, which is not surprising given that the complex sensory and cognitive demands of foraging are not necessary for them.

Queens do have stingers that are important to their biology, but this biology is distinct from that of workers. Behaviorally, queens do not defend the nest. They use their stings only to fight other queens, or to kill them in their cells prior to emergence (Gilley, 2001). Their stings are thus not barbed (the barb is useful against vertebrates). The queen's venom sac is also full upon emergence, unlike the worker's, which only gradually fills as it ages. Studies calculating the LD_{50} (the concentration of compound killing half of exposed individuals) for worker versus queen venom have shown that queen and worker venom are different in nature, which is adaptive given that the worker's venom must be effective against vertebrates and invertebrates, while the queen's is only used against bees (Schmidt, 1995). Hyaluronidase, in particular, which

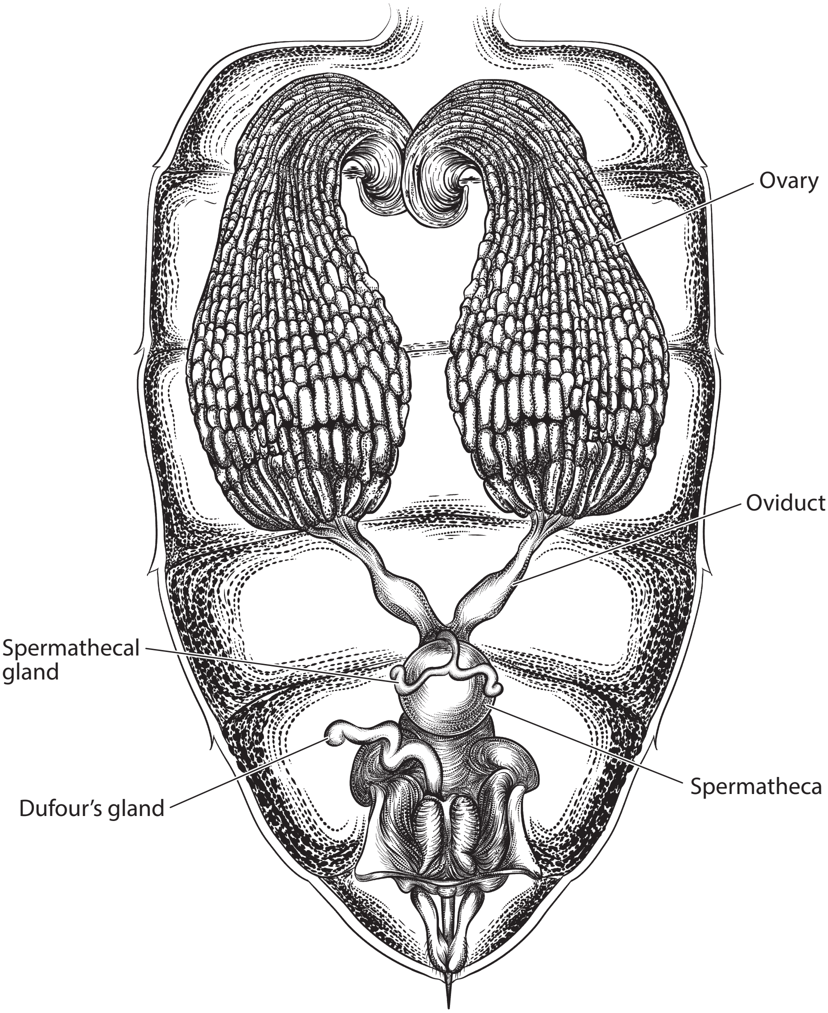


FIGURE 4.15. Queen reproductive system (*redrawn from Snodgrass, 1956*).

helps break down dense vertebrate skin, is lower in concentration in queen versus worker venom (Owen, 1979). Given that the queen is unlikely to use her stinger again once she mates, it is also not surprising that her venom is allowed to degrade over time, and many old queens have no functional venom (Owen and Bridges, 1976).

The most obvious and important internal difference between queens and workers relates to the reproductive system, which is hugely expanded in queens and equally reduced in workers, even when laying (figure 4.15). Queens have two lateral ovaries, each comprised of 150–180 ovarioles. They

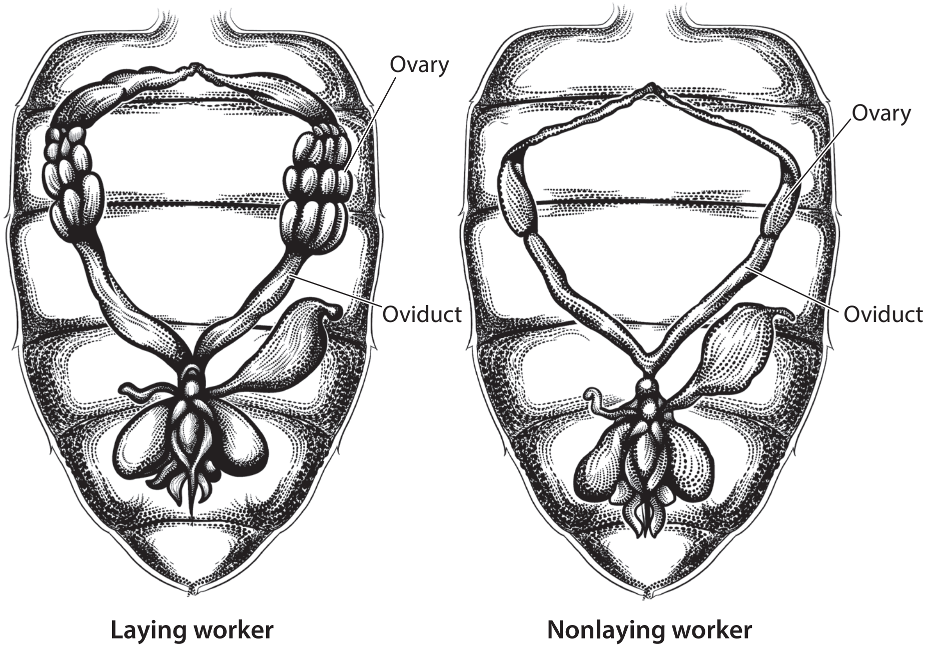


FIGURE 4.16. Laying and sterile worker reproductive systems (*redrawn from Snodgrass, 1956*).

are capable of laying over a thousand eggs per day (Allen, 1960, Hartfelder and Steinbruck, 1997). Queens also have a sperm storage organ, called the spermatheca, capable of holding about five to seven million sperm, along with associated glands for keeping the sperm healthy. Queens mate early in life and never again, so once the spermatheca runs out, the queen is superseded by the colony. Queens also have the physiological ability to fertilize, or not fertilize, an egg as it passes down the oviduct. This is accomplished with a pump and valve associated with the spermatheca that allows the queen to release only a few sperm at a time (Ruttner, 1956b). The worker reproductive system, in contrast, is quite simple. Workers have only 2–8 ovarioles, and their spermatheca is vestigial and nonfunctional (figure 4.16). Workers thus cannot mate and are only capable of laying unfertilized eggs.

Drone Anatomy and Physiology

Drones differ in many ways from workers and queens. Most of these differences are associated with their simple life history. Drones do not work in the nest, so it is not surprising that they do not have pollen baskets, for example, or most of the glands associated with social behavior that we have discussed (Snodgrass, 1956). Their behavior consists of flying to drone congregation areas and attempting to mate with virgin queens. The act of mating leads to their almost immediate death, as the endophallus, or intromittent organ, which is quickly everted from the bee's body during mating, breaks off, and remains inserted in the queen's vaginal cavity.

In keeping with their life history, drones have powerful flight muscles, large eyes, and a reproductive system that is capable of rapidly inseminating queens in flight with a large quantity of semen (Winston, 1991). Their antennae are also more sensitive than those of workers, with about ten times the number of plate organs, the structures underlying olfaction (Esslen and Kaissling, 1976, Brockmann and Bruckner, 2001). The drone reproductive system, with its claspers for grasping the queen in fight, the endophallus, testis, and associated glands, are discussed (and shown) in chapter 8 in the context of mating.

Honey Bee Nutrition

Honey bees, like most bees, are herbivores, obtaining all their nutrients, as both larvae and adults, from plants. Pollen is the main protein and lipid source and nectar the main carbohydrate source. In highly social bees, like honey bees, long-term storage of plant products as honey (concentrated nectar) and bee bread (stored pollen preserves) is a fundamental part of life.

In general, how honey is made is relatively well understood, while the making of bee bread is less well understood.

Nectar and Honey

Nectar is the colony's source of carbohydrates (Brodschneider and Crailsheim, 2010). Nectars vary widely in their contents, with the percentage of sugar ranging from about 5% to about 80% (White, 1975). Most nectars are in the range of 20%–40% sugar. Fructose, glucose, and sucrose are the primary sugars in nectar, and the percentage of each varies widely across plant species (Doner, 1977). Larger sugars are also present and are important for giving honey its unique flavor. Maltose, trehalose, and melezitose are common examples of sugars that are digestible by bees. Nectar also contains some amino acids, vitamins, minerals, and ash, which gives darker honeys their color (Haydak, 1970, Wright et al., 2018). These are not thought to be essential for bee health, as pollen is the main source of vitamins and minerals. There is also evidence that bees seek out water sources with high levels of some vital vitamins and minerals (Lau and Nieh, 2016, Bonoan et al., 2017, 2018). Honey bees also sometimes collect honeydew from plant-sucking insects. Typically, honeydew is a minor component of honey, which is fortunate, as it seems to cause dysentery at high concentrations. Finally, bees visit extrafloral nectaries when they are available.

Honey is the result of two main activities by the bees. First, digestive enzymes are added to nectar beginning in the crop of the forager on her way back to the nest (Nicolson and Human, 2008). Enzymes—chiefly invertase, diastase, and glucose oxidase—break down larger sugars into simpler sugars and make the honey more acidic and therefore less prone to microbial attack (White and Maher, 1953, White et al., 1963, Wright et al., 2018). Second, the middle-age bees, who receive the nectar from foragers, dehydrate it down to less than 18% water, making it a hostile place for microbial growth. This, of course, also shrinks the volume of the nectar for more efficient storage. Freshly made honey is quickly capped and in this state is viable for many years.

Pollen and Bee Bread

Pollen is the bee's source of protein, lipids, vitamins, and minerals (Haydak, 1970, Crailsheim et al., 1992). As for nectar, pollen from different plant sources is quite variable. Protein content, for example, which has received the most attention, varies from about 10% to about 60% (Herbert and Shimanuki, 1978, Wright et al., 2018). Bee bread is typically in the range of 10%–30% protein (Human and Nicolson, 2006). More important than raw concentration, however, is the concentration of various amino acids (Schmidt et al., 1987, 1995, Roulston and Cane, 2000). Bees have 10 essential amino acids that they need in particular amounts from their diet (Degroot, 1952). The most limiting are leucine, isoleucine, and valine (Brodschneider and Crailsheim, 2010). In nature, bees collect pollen from many sources, creating a balanced combination. Should too much pollen stem from a single source, however, it can cause malnutrition (Herbert et al., 1970, Loper and Berdel, 1980, Schmidt et al., 1987, Alaux et al., 2010b). Bees are good at extracting protein from pollen, as they extract about 75% of the protein content during digestion (Schmidt and Buchmann, 1985). In contrast, they are far less efficient at digesting the pollen in artificial feeds (DeGrandi-Hoffman et al., 2016).

A similar picture is present for lipids, and the importance of balanced lipid consumption has received increasing attention in recent years. Pollen grains range from 7% to 20% lipids, while bee bread averages about 3%–8% (dry weight) (Roulston and Cane, 2000, Wright et al., 2018, Arien et al., 2015). In general, the lipids on the surface of the pollen grains tend to be antimicrobial (a function beneficial to the plant, but also probably to the bees) while the inner portion is more nutritious (Manning, 2001). As for proteins, there are essential and nonessential fatty acids (Roulston and Cane, 2000). Both are necessary, but the essential fatty acids must be acquired in the diet as they cannot be endogenously synthesized. The primary essential fatty acids for bees are alpha-linolenic acid (an omega-3) and linoleic acid (an omega-6). Recent work has shown the balance between the two may be key to health, as bees with an imbalance (too high a ratio of omega-6 to omega-3) suffer learning and memory deficits (measured by the PER bioassay) and low survivorship as brood and adults (Arien et al., 2015, 2018, 2020). The two essential fatty acids, plus palmitic acid, make up the bulk of lipids in pollen. Pollen also contains sterols, which are necessary for the production of ecdysone, and other bodily functions (Svoboda et al., 1980).

Bee bread has a lower pH and less starch than fresh pollen (Herbert and Shimanuki, 1978, Ellis and Hayes, 2009). The older literature suggested that bee bread was more nutritious than most sources of fresh pollen (Hagedorn and Moeller, 1968, Herbert and Shimanuki, 1978, Pernal and Currie, 2000) and argued that processing of pollen into bee bread was the cause. More recent work has questioned this perspective. Anderson et al. (2014) found that older bee bread is not preferred over recently made bee bread, and that little functional processing by microbes occurs in bee bread (figure 4.17). They argue that the function of bee processing is simply to preserve the pollen and protect it from microbial breakdown. In support of this, it was shown that bees prefer to consume freshly stored pollen (Carroll et al., 2017) and that feeding on aged pollen leads to nutritional imbalance (Maes et al., 2016).

Brood Food (Worker and Royal Jelly)

Honey bees differ from nearly all other bees by feeding their young a glandular secretion, rather than pollen mixed with nectar directly. Thus, the nutrition of the larva is entirely dependent on bodily secretions, and only indirectly on pollen and nectar. Much effort has gone into identifying the contents of brood food, which is secreted by the nurse bee's mandibular and hypopharyngeal glands (Haydak, 1943, 1970). In general, royal jelly is about 63% water, 18% carbohydrates, 14% protein, and 6% fats (Wright et al., 2018). It also contains

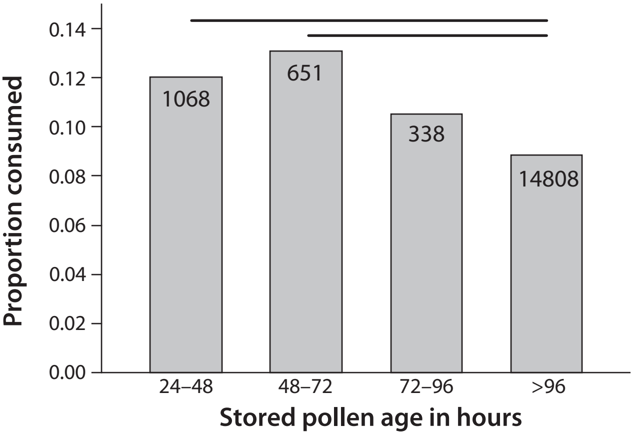


FIGURE 4.17. Consumption of stored pollen by age (*from Anderson et al., 2014*).

sterols and all the micronutrients needed for growth and health (Lercker et al., 1982). The carbohydrates in brood food are the same as in honey, while the protein is entirely different. The major royal jelly proteins (covered in chapters 3 and 9) make up the bulk of the protein in brood food. We covered the controversial question of whether they have a role in caste determination (as signals, rather than as food) in chapter 3. For our purposes here, they almost certainly serve a fundamental role as a simple protein source. Finally, it must be stressed that adult workers, drones, and the queen all acquire their protein and lipids from jelly, as the nurses pass half of their brood food to adults (Crailsheim, 1990, 1991, Crailsheim et al., 1992). The names "brood food" and "royal jelly" are thus misnomers of a sort in that they incorrectly imply that only the young (or queens) are fed these vital secretions.

Foods Toxic to Bees

Some sources of nectar and pollen are toxic to bees (Barker and Lehner, 1974, Barker, 1990). In nature, this is rarely a problem as these food sources are highly diluted when mixed with food from many other plants. With respect to nectar collection, bees avoid most, but not all, of these plants as the nectar is usually bitter. Occasionally, however, when bees are kept in a location in which forage is only available from a few plants and one or more is toxic, the bees (or humans who eat the honey) can suffer. In general, nectar is often toxic because it contains harmful compounds like toxic alkaloids, while pollen is typically toxic because it contains sugars that bees cannot digest (Barker, 1977). Toxic sources of nectar include oleander, azalea, azure, black hellbore, California buckeye, and jimsum weed. Some toxic sources of pollen include California buckeye, balsa, and flame of the forest.

The Microbiome

Historically, it was difficult to get an accurate picture of the microbiota living in organisms, as some microbes cannot be cultured and the large diversity of species makes PCR-based methods impractical for discovery of community richness (Cho and Blaser, 2012, Kwong and Moran, 2016). Next-generation sequencing allows one to sequence everything in a sample, however, which allows for the cataloging of microbial diversity. This has led to an enormous surge in studies of the microbiome (Kwong et al., 2017b, Anderson and Ricigliano, 2017, Raymann and Moran, 2018).

Larval Microbiome

The larval microbiome is less diverse and more variable than that of adults (reviewed in Kwong and Moran, 2016). First and second instar larvae primarily contain *Acetobacteraceae* Alpha 2.2 (*Bombella apis*) in their guts, likely originating in worker jelly (Vojvodic et al., 2013). This species is highly derived when associated with bee species that nurse their young (Corby-Harris et al., 2014b). Later larval instars have a microbiota composed mainly of *Lactobacillus* spp. (Vojvodic et al., 2013).

Adult Worker Microbiome

Newly emerged bees have a depauperate microbiome (Kwong and Moran, 2016). The larval gut is shed during pupation, and the newly emerged bee starts life mostly free of microbes. Within a few days, however, they are inoculated with microbes they receive from the nurses (orally and perhaps via fecal material), and from the nest environment (pollen and comb). By day four, the relatively stable adult microbiome is in place (Powell et al., 2014). In the process of microbial colonization, the first behaviors performed by newly emerged bees—cell cleaning and consumption of pollen—may be critical (Anderson et al., 2016). These behaviors are performed inflexibly upon emergence (with stereotyped regularity), unlike most tasks (Johnson, 2010a), and it has been

Table 4.2. Common honey bee microbiota

Species	Core?	Caste/Developmental stage	Location in the body
Lactobacillus Firm 4	Yes	Worker, queen, drone	Rectum
Lactobacillus Firm 5	Yes	Worker, queen, drone	Rectum
Snodgrassella alvi	Yes	Worker	Ileum
Gilliamella apicola	Yes	Worker	Ileum
Bifidobacterium sp.	Yes	Worker	Rectum
Frischella perrara	No	Worker	Pylorus
Bombella apis	No	Worker, larva, queen	Worker crop, queen and larval gut
Bartonella apis	No	Worker	Gut
Acetobacteraceae	No	Worker, larva, queen	Crop, HP glands, larval gut

shown that the core microbiota would be encountered in these contexts (Anderson et al., 2014, Corby-Harris et al., 2014b). Further, Anderson et al. (2016) showed that interactions with the hive materials alone lead to the establishment of hindgut microbiota. This occurs with or without the presence of older workers. This work also suggests that the establishment of the microbiota is akin to ecological succession, as early colonizers give rise to a stable community in a predictable but variable fashion.

The microbiome in nurses and older bees is composed of a small number of taxonomically restricted clades, some of which are common to bees and some of which are unique to honey bees (Jeyaprakash et al., 2003, Babendreier et al., 2007, Martinson et al., 2011, Sabree et al., 2012). Table 4.2 lists the most common species, which consist of five species clusters found in most samples plus a small number of others with more variable patterns of presence or absence. The microbes are characterized by a spatial pattern within the bee's body. The crop contains few microbes, most of which also occur in the nest and in nectar (Martinson et al., 2012, Corby-Harris et al., 2014a). *Lactobacilus kunkeei* and *Bombella apis* are key members of the crop community. The midgut contains few microbes, other than pathogenic ones in sick bees, and the hindgut (ileum and rectum) is where the main microbiome resides. Microbes within the hindgut are further spatially segregated into those that reside in the ileum (gram-negative species) and the rectum, where the fermentative gram-positive microbes, such as *Lactobacillus* sp., reside. The microbes in the hindgut form a structured (with respect to species and their location) biofilm (Martinson et al., 2012).

The work on the microbiome(s) is mainly based on 16S rRNA sequencing, which allows one to classify microbes taxonomically but not functionally. Engel et al. (2012) conducted a metagenomics study in which all microbial DNA was sequenced and found evidence for much functional diversity. *Diversity* here refers to proteins that serve many metabolic and structural roles relating to different microbial life history strategies. In other words, this work suggested that the taxonomic simplicity of the bee microbiome is probably not indicative of functional simplicity, and it may not even be indicative of species-level diversity if a few clades turn out to contain a great many distinct species.

Follow-up studies using shotgun sequencing have validated the idea that the 16S rRNA results hide much functional (and strain or species-level) diversity. In short, important taxonomic and functional diversity has been found for all five core microbial strains (Ellegaard et al., 2015, 2020, Kesnerova et al., 2017, Ellegaard and Engel, 2018, 2019, Raymann et al., 2018, Bobay et al., 2020, Smith et al., 2021). Some strains of *G. apicola*, for example, can metabolize sugars toxic to bees, while strains of *S. alvi* with greater resistance to glyphosate have been found (Zheng et al., 2016, Motta et al., 2018). It is also apparent that the microbiota across species and individuals is more variable than previously thought (Ellegaard et al., 2020, Ellegaard and Engel, 2019). Old bees, in particular, are more likely to be colonized by noncore microbes at high levels (Ellegaard and Engel, 2019).

Queen and Drone Microbiomes

The queen and drone microbiomes are simpler than those of the workers and are more variable across individuals (Raymann, 2021). In short, queens typically possess *Bombella apis*, *Acetobacteraceae*, *Lactobacillus* Firm-4, and *Lactobacillus* Firm-5, while the drones have *Lactobacillus* Firm-4 and *Lactobacillus* Firm-5 (Tarpy et al., 2015, Kapheim et al., 2015b, Anderson et al., 2018, Powell et al., 2018). In general, bacteria that dominate the queen gut are also associated with worker hypopharyngeal glands (Corby-Harris et al., 2014b), used by nurses to feed the queen, which may be their source. There is also some evidence that the queen microbiome changes with age, with enteric bacteria being dominant early in life and alpha proteobacteria being dominant at maturity (Tarpy et al., 2015, Anderson et al., 2018). It is not clear if these age-related changes are adaptive, however, as they could be the result of changes that occur within the bacterial community that are functionally neutral to the queen.

Nest Microbial Community

The hive environment (the wax, honey, and pollen) contains a large diversity of microbial life (Anderson et al., 2013, Corby-Harris et al., 2014b). The core microbiota are found in these places, but as these environments, which are often sugary and acidic, are quite different from the hindgut, there is incomplete overlap between the microbes residing in these areas and the core microbiota. *Bombella apis,* for example, is found in the crops, hypopharyngeal glands, and royal jelly but not in the worker hindgut (Corby-Harris et al., 2014b). *Bombella apis* may also serve a protective role by limiting the growth of pathogens (Corby-Harris et al., 2016, Miller et al., 2021).

Function of the Microbiome

In general, the microbiomes of insects tend to serve a limited number of functions (Kwong and Moran, 2016, Raymann and Moran, 2018). Most commonly, either the microbes provide some metabolic capacity that the insect lacks, or nonpathogenic microbes are encouraged to colonize vulnerable places within the insect to prevent the establishment there of harmful species (F. J. Lee et al., 2015). For bees, the primary idea regarding metabolism is that the microbes ferment sugars the bees cannot, and excrete short fatty acids the bees can then utilize (Lee et al., 2018). With respect to microbial symbiont regulation, in bees this would extend from the body to include the nest (Anderson et al., 2013, 2016). Antimicrobial propolis, for example, is used to regulate the nest microbiome (Saelao et al., 2020, Dalenberg et al., 2020). Overall, there is evidence for both forms of adaptive benefit in the honey bee.

With respect to providing increased metabolic capacity, it has been suggested that microbes could help in the breakdown of pollen (Engel et al., 2016). Plant cell walls are difficult to metabolize, as they contain several compounds that are resistant to digestion (Watanabe and Tokuda, 2010). Cellulose, the main component, can be broken down with endogenous cellulases, but bees, like most insects, lack enzymes to break down pectin. Engels et al (2012), in their metagenomic study of honey bee functional protein diversity, identified bacterial pectinases, which could be useful for breaking down plant cell walls to release nutrients for absorption. Later studies also suggest that bee gut symbionts could be involved in digestion (Kesnerova et al., 2017, Ellegaard and Engel, 2016, Lee et al., 2018). In a study utilizing microbe-free bees, for

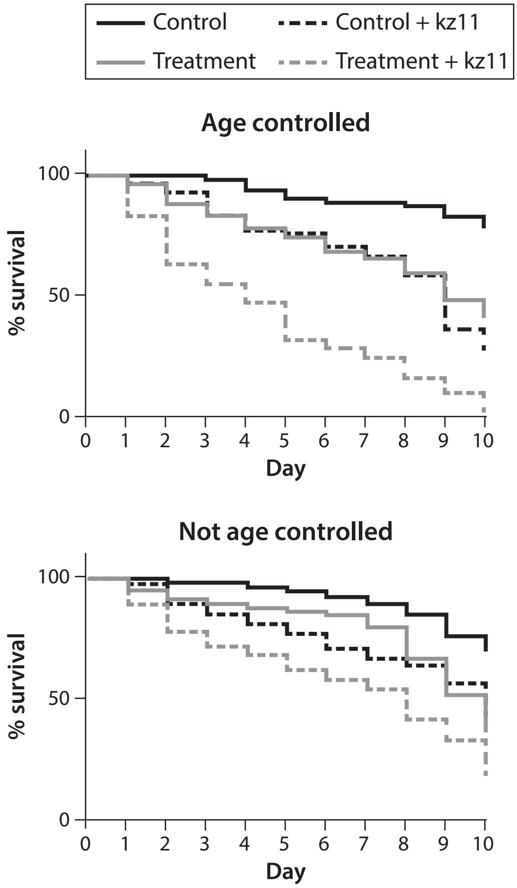


FIGURE 4.18. Survivorship of bees treated with antibiotics (treatment). Treated bees had decreased survivorship when challenged by an opportunistic pathogen, *Serratia* (kz11) (*from Raymann et al., 2014*).

example, Zheng et al. (2017) showed faster weight gain of bees with the normal microbiota. What causes this effect remains unclear.

The question of whether the microbiome plays a role in defense against pathogenic microbes has also received some attention. Forsgren et al. (2010) showed that microbes from the crop (*Lactobacillus* and *Bifidobacterium*) inhibit *Paenibacillus larvae*, the bacterium causing American foul brood, on petri dishes. Schwarz et al. (2016) inoculated newly emerged bees with *Snodgrassella alvi*, a member of the core microbiome, and found a strong effect on the mature microbiome such that inoculated bees eventually formed a depauperate microbiome and were more susceptible to *Lotmaria passim*, a protozoan parasite. This suggests the totality of the microbiome (the whole community) may be important for bee health.

There are also now a growing number of studies showing general health benefits associated with the microbiota. Many of these studies make use of the fact that newly emerged bees are relatively microbe-free. This allows one to rear clean bees in an incubator, which can later be inoculated with microbes. The use of antibiotics to eliminate microbes in older bees has also been productive. *Frischella perrara*, for example, colonizes the pyloris between the midgut and the hindgut, causing a melanized scab to form (Engel et al., 2015). This bacterium was shown to trigger an immune response in microbe-free bees challenged with it (Emery et al., 2017). This may prime the immune system for subsequent challenges by harmful microbes. Raymann et al. (2017) showed that antibiotics (commonly used to treat larval diseases) affect normal microbiome composition, which lowers bee survivorship when challenged by opportunistic pathogens (figure 4.18). Kwong et al. (2017a) showed that bees with a microbiome are better able to survive infection by *Escherichia coli*, perhaps because of immune priming.

5

Genetics and Genomics

In this chapter, we focus on the genetic basis of social traits and provide a description of the honey bee genome. The studies we review mainly explore the genetic basis of traits in the classical genetics sense, which is to say they focus on the genetic basis of phenotypic variation. Much of the research is associated with a large body of work exploring the genetics of pollen storage (Hunt et al., 1995a, 1995b, Page and Fondrk, 1995, Page et al., 2000, 2006, 2012, Ruppell et al., 2004, Graham et al., 2011). This involves many individual- and colony-level traits associated with forager preferences, food storage, and usage. We emphasize quantitative trail loci (QTL) and artificial selection work and leave discussion of downstream functional studies to chapter 15 on foraging. Genetics work associated with development, neurobiology, division of labor, and several other topics are likewise covered in the chapters addressing those issues.

Classic Studies in Honey Bee Genetics

Early work in genetics was focused on identifying simple Mendelian traits. These are traits that exhibit variation that falls into discrete classes. Mendel's work on wrinkled versus smooth peas is a good example. This work often involved the identification of phenotypic mutants, which were then inbred and crossed to reveal diagnostic ratios. Fruit fly studies in this context are well known to all introductory biology students. Not nearly so well known is the literature on honey bees in this paradigm. Table 5.1 lists the honey bee mutants found to have simple Mendelian genetic bases. A few of these traits were found to be linked (reviewed in Rothenbuhler et al., 1968). This work has largely not been continued, but we reference it here because the arrival of new techniques sometimes makes intractable problems amenable to study and some of these mutants could be useful.

Table 5.1. Classic Mendelian honey bee mutations

Mutation name(s)	Trait affected
Brick, chartreuse, benson green, cherry, red, cream, garnet, ivory, umber, pink, pearl, snow, tan	Eye color
Cordovan, black	Body color
Droopy, rudimental wing, short, truncate, wrinkled	Wings
Eyeless	Eye morphology
Hairless	Body hair
Removing, uncapping	Behavior

Source: Rothenbuhler et al., 1968.

Behavioral Genetics Based on Inferred Genotypic Differences

In contemporary genetics studies, the goal is to explicitly link genes (or DNA sequences) with phenotypes. Strictly speaking, however, it is not necessary to know which genes underlie a trait to show that it has a genetic basis. Studies of heritability, of course, infer the genetic basis of traits based on patterns of resemblance between relatives. Some key studies showing genetic bases for important behavioral traits in bees also used a simple approach based on relatedness, specifically differences in behavior across patrilines. Patrilines, or subfamilies, are all those bees in a colony who share a father. Because queens mate 12 times on average, there are usually at least 12 different patrilines in a nest (Tarpy and Page, 2001). If workers in different patrilines vary for a behavior, there is a high likelihood that the trait is genetically determined to some extent. This work was pivotal for the field, as it led to several high-profile papers that triggered intense interest in bee behavioral genetics.

The study that pioneered this work showed that high- and low-strain pollen hoarding bees (discussed later), when reared in the same nest, differ in their probability of collecting nectar versus pollen, as well as in their age at first foraging (Calderone and Page, 1988). This study led to many dozens of follow-up studies in wild-type colonies (Robinson and Page, 1988, Withrow and Tarpy, 2018, Mattila and Seeley, 2011, Palmer and Oldroyd, 2003, Calderone and Page, 1991, Page et al., 1998, Frumhoff and Baker, 1988, Oldroyd et al., 1993, Kolmes et al., 1989). In these studies, genetic markers—usually microsatellites—were used to genotype bees and assign them to patrilines. Biases in the frequencies of bees from different patrilines across different tasks then suggested that genotype affects task choice. Key studies showed genotypic effects on guarding and undertaking, among other tasks. This work triggered much of the interest in the stimulus response model for task allocation, which we cover in chapter 13.

Quantitative Genetics Studies in Bees

Most phenotypic traits do not fall into qualitatively discrete categories like Mendel's peas. Height, reaction time, body color, many behavioral preferences, and so forth all show more or less continuous variation (Collins, 1986). The genetic basis of such traits depends on the effects of many genes. Classically, the study of continuous traits has fallen under the umbrella of quantitative genetics (Falconer and Mackay, 1996).

Heritability Studies

Heritability, in the narrow sense, is the ratio of additive genetic variance to total phenotypic variance in a particular population (Falconer and Mackay, 1996). It can be used to predict the response to selection, or the breeding value of individuals, and it also can be used (with caution) to infer how much of the variation for a trait is due to genetic differences. As for the Mendelian studies in honey bees, there is a large, older body of literature on estimating heritability for honey bee traits (Collins, 1986). Some of these results are shown in table 5.2. Many social traits have heritability scores of a size comparable to that found for commonly studied quantitative traits, such as height or weight. These traits therefore respond to selection, and there is also an older body of literature on using artificial selection to improve bees for traits such as honey production or plant preference (Rothenbuhler, 1958, Rothenbuhler et al., 1968). Heritability is still sometimes calculated in contemporary honey bee studies (Oldroyd and Moran, 1983, Le Conte et al., 1994a, Goudie et al., 2012), but it is not an active area of research at the moment.

QTL Primer

The basic principles of QTL mapping are illustrated in figure 5.1 (Oldroyd and Thompson, 2007). Two genetic lines that differ strongly for the phenotype of interest are used. These can be naturally occurring subpopulations, or they can be produced via artificial selection. When artificial selection is used, this is

Table 5.2. Heritability values for honey bee traits

Trait	h2 score
Honey weight (various seasons)	0.16–0.6
Brood rearing (various seasons)	0.30–0.76
Hamuli number	0.68–0.76
Reaction time to isopentyl acetate	0.03–0.68
Reaction time to moving target	0.69
Number of bees responding to disturbance	0.17
Pupal weight	0.645
Corbicular area	1
Longevity	0.2–0.32
Postcapping stage	0.68

Source: Collins, 1986.

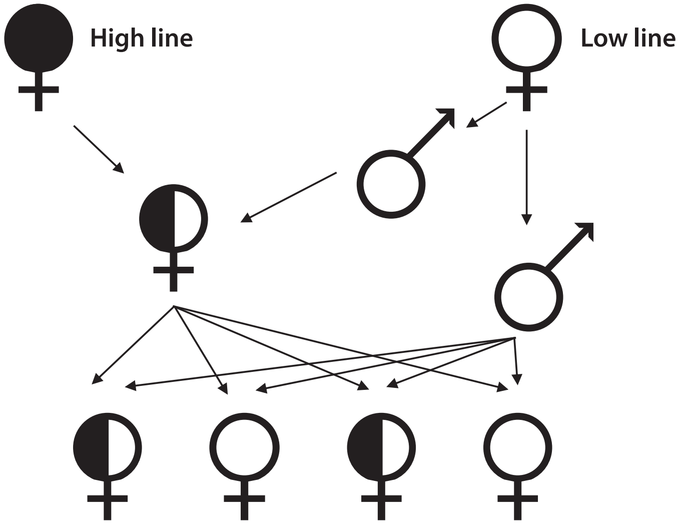


FIGURE 5.1. Basic experimental approach to identifying QTLs in bees (*redrawn from Oldroyd and Thompson, 2007*).

often coupled with inbreeding to increase the homogeneity of individuals in the lines. Individuals from the two lines are crossed to produce an F1 hybrid queen that is presumably heterozygous for the genes contributing to the difference in phenotype. The F1 queens are then backcrossed to one or both lines (usually the line with the recessive, or less dominant, phenotype). The progeny of the backcross is thus composed of both homozygotes for the genes contributing to the low line and heterozygotes in more or less equal frequencies. These individuals are then phenotyped and genotyped at a large number of neutral molecular markers (for which a linkage map has been produced) spread across the genome. If a QTL is present near a marker, then the homozygous individuals will differ in their mean phenotype from those that are heterozygous at that locus. If the backcross is to the low line, for example, then homozygotes will have lower scores relative to heterozygotes. Each locus could be tested one by one, but this approach is weaker than testing multiple loci at once using sophisticated statistical techniques that allow one to better narrow down where the gene contributing to the QTL is likely located. This is called interval mapping.

Honey Bee QTL Studies

POLLEN HOARDING

One of the largest bodies of experimental work on honey bees has been conducted by Rob Page and Greg Hunt and collaborators on the social organization and genetic basis of pollen storage (reviewed in Page et al., 2012). This research built on pioneering work in the 1980s by Rothenbuhler and colleagues selecting for colonies that stored low and high levels of pollen (Hellmich et al., 1985). The later work identified many QTLs underlying this phenotype and also led to studies on other traits involving reproduction that were found to affect foraging choices (table 5.3). Here we review the purely genetic work, leaving the behavioral and physiological details for other chapters.

Artificial selection with inbreeding was used to produce high and low pollen storing lines of bees (Page and Fondrk, 1995). Following many generations of selection, the high line stored six times the pollen as the low line. These bees stored so much pollen, in fact, that they left little to no room for egg laying. The researchers identified many other phenotypes that responded to selection for this colony-level trait (Page et al., 2012). Chief among these are variation at the age of first foraging, changes to development times, variation in the number of ovarioles, and variation in sucrose responsiveness. The number of correlated responses suggested that a complex genetic basis involving epistasis and pleiotropy likely underlies pollen storage.

The first QTL study using the high and low lines identified two QTLs, which were named pln1 and pln2 (Hunt and Page, 1995, Hunt et al., 1995b). Two

Table 5.3. QTL studies growing from work on pollen hoarding bees

Trait	Map population	Effects and interactions	References
QTL (Chromosome: MB): *pln1* (13:3.5)			
Pollen hoarding	HBC	Direct	Hunt et al., 1995a
Pollen load size	HBC	Direct x pln2 x pln3	Hunt et al., 1995b, Rueppell et al., 2004a
Nectar load size	HBC	Direct	Hunt et al., 1995b
Pollen proportion	LBC	x pln3 x pln2 x pln3 x pln4	Rueppell et al., 2004a
Nectar concentration	LBC	x pln2 x pln3 x pln4	Rueppell et al., 2004a
Sucrose responsiveness	HBC, LBC, HXL, hybrid males	Direct	Rueppell et al., 2006
Age first foraging	HBC, LBC	Direct x pln 3 x pln2 x pln3	Rueppell et al., 2004b
Worker ovary size	ABC, EBC	Direct	Graham et al., 2011, Ihle et al., 2015b
QTL (Chromosome: MB): *pln2* (1:16.5)			
Pollen hoarding	HBC, EBC	Direct	Hunt et al., 1995a, Page et al., 2000
Pollen load size	HBC	Direct x pln4 x pln1 x pln3 x pln3 x pln4	Hunt et al., 1995b, Rueppell et al., 2004a, Page et al., 2000
Nectar load size	HBC	Direct x pln4	Hunt et al., 1995b, Rueppell et al., 2004a
Pollen proportion	HBC, LBC	Direct x pln4 x pln1 x pln3 x pln4	Hunt et al., 1995b, Rueppell et al., 2004a
Nectar concentration	HBC, LBC	Direct x pln1 x pln3 x pln4	Hunt et al., 1995b, Rueppell et al., 2004a
Sucrose responsiveness	HBC	x pln3	Rueppell et al., 2006
Age first foraging	LBC	x pln1 x pln3	Rueppell et al., 2004b
Worker ovary size	HBC	Direct	Wang et al., 2009
Worker ovary size	ABC	Direct	Graham et al., 2011
QTL (Chromosome: MB): *pln3* (1:9.2)			
Pollen hoarding	HBC	Direct	Page et al., 2000
Nectar load size	HBC	Direct	Page et al., 2000
Pollen load size	HBC	Direct x pln1 x pln2 x pln2 x pln4	Page et al., 2000, Rueppell et al., 2004a
Pollen proportion	LBC	x pln 1 x pln1 x pln2 x pln 4	Rueppell et al., 2004a

Continued on next page

Table 5.3. (*continued*)

Trait	Map population	Effects and interactions	References
Nectar concentration	HBC, LBC	Direct x pln4 x pln1 x pln2 x pln4	Page et al., 2000, Rueppell et al., 2004a
Sucrose responsiveness	HBC, LBC	x pln2 x pln4	Rueppell et al., 2006
Age first foraging	LBC	x pln1 x pln1 x pln2	Rueppell et al., 2004b
Worker ovary size	HBC	Direct	Wang et al., 2009
QTL (Chromosome: MB): *pln4* (13:9.0)			
Nectar load size	HBC	x pln2	Rueppell et al., 2004a
Pollen load size	HBC	x pln2 x pln2 x pln3	Rueppell et al., 2004a
Pollen proportion	HBC, LBC	Direct x pln2 x pln1 x pln2 x pln3	Rueppell et al., 2004a
Nectar concentration	HBC, LBC	Direct x pln3 x pln1 x pln2 x pln4	Rueppell et al., 2004a
Sucrose responsiveness	HBC, LBC	Direct x pln3	Rueppell et al., 2006
Worker ovary size	HBC	Direct	Ihle et al., 2015b
QTL (Chromosome: MB): *aff2* (11:13.1)			
Age first foraging	HBC	Direct	Rueppell et al., 2004b, Rueppell, 2009
Worker ovary size	ABC	Direct	Graham et al., 2011
QTL (Chromosome: MB): *aff3* (4:9.1)			
Age first foraging	LBC	Direct	Rueppell et al., 2004b, Rueppell, 2009
QTL (Chromosome: MB): *aff4* (5:8.8)			
Age first foraging	LBC	Direct	Rueppell et al., 2004b, Rueppell, 2009
Worker ovary size	ABC	Direct	Graham et al., 2011
QTL (Chromosome: MB): *was1* (3:13.1) & *was2* (2:10.7)			
Worker ovary size	HBC	Direct	Rueppell et al., 2011, Ihle et al., 2015b
QTL (Chromosome: MB): *was3* (4:1.8)			
Worker ovary size	LBC	Direct	Rueppell et al., 2011, Ihle et al., 2015b
QTL (Chromosome: MB): *was4* (11:10.7) & *was5* (6:14.2)			
Worker ovary size	ABC, HBC	Direct	Graham et al., 2011, Ihle et al., 2015b

Source: Page et al., 2012.

follow-up studies using crosses of Africanized and European bees, and the original lines following more selection, identified two more QTLs, pln3 and pln4 (Page et al., 2000, Ruppell et al., 2004). A number of follow-up QTL studies verified the original results, an important need in QTL studies as the results are sometimes population specific. Follow-up studies on other traits, discussed shortly, demonstrated pleiotropic effects and epistatic interactions.

The completion of the honey bee genome allowed for the identification of candidate genes in the identified QTLs. In general, the pollen QTLs are not terribly large, but they are large enough to make identification of the relevant nucleotide differences difficult. The range of candidate genes found in the QTLs, for example, ranged from 4 to 59, and this is just using the official annotation of the genome. Given that many unannotated genes likely also exist in the regions, the number of candidates could be larger. Nevertheless, a bias of genes in the insulin pathway in the pln QTLs suggested that this important pathway, involved in nutrient signaling, may partly determine foraging choices (Hunt et al., 2007). The change in response threshold to sucrose in the proboscis extension reflex bioassay identified early in the pollen hoarding bees also supported the notion that insulin signaling may be involved (Page et al., 1998).

One candidate gene in pln2, a tyramine receptor (*TYR1*), was the focus of a recent study validating its role in pollen storage (and social behavior in general). Tyramine (*TYR*) is an important neuromodulator associated with the control of behavior in many systems. It is also the precursor for octopamine, another important neuromodulator known to regulate honey bee behavior (see chapter 12). When Wang et al. (2020) fed *TYR* to developing larvae, they found an association between higher *TYR* titer and more ovarioles. *TYR* feeding also affected ovarian activation in adults. They then showed that knockdown of *TYR1*, via RNAi, affected the expression of vitellogenin and several other genes known to underlie division of labor.

Two correlated responses to selection for pollen hoarding, variation in the age at first foraging and variation in ovary size, led to follow-up QTL studies. Age at first foraging differs between several populations of honey bees (high and low pollen hoarding, and European and Africanized bees), and a QTL study making use of these populations found four QTLs for this trait (Rueppell et al., 2004). This study also confirmed a role for pln1 for determining age at first foraging, showing a pleiotropic effect, assuming the same gene in the QTL is responsible in both contexts. Studies using the high and low pollen hoarding strains and Africanized and European bees were also used, along with denser molecular markers, to identify three QTLs for variation in ovariole number (Linksvayer et al., 2009b, Wang et al., 2009, Graham et al., 2011, Rueppell et al., 2011). This work showed that one of the pln QTLs was associated with ovariole number. In fact, over the course of many studies, several interactions between the new and original pollen hoarding QTLs were found, suggesting a complex genetic basis for these linked traits (Rueppell et al., 2006, Ihle et al., 2015b). This nexus of overlapping traits with a common genetic basis was named the pollen hoarding syndrome. This syndrome is thought to link variation in reproductive biology with the evolution and control of social foraging in bees (Page et al., 2012). Much work followed up on these studies, which is reviewed in the chapters on evolution (chapter 9), division of labor (chapter 12), and foraging (chapter 15).

DEFENSIVENESS (AGGRESSION)

Defensiveness is one of the bee's core adaptations and a trait of great interest to humans for obvious reasons. Its study has a long history that increased dramatically in scope with the spread of Africanized bees in the Americas. A classic QTL study using RAPD markers, following closely the methods used in the pollen hoarding studies, was conducted on honey bee defensiveness (Hunt et al., 1998). The two lines were European and Africanized bees from the same location in Mexico. Africanized drones were crossed to European queens using artificial insemination. Backcrosses of these F1s were then done using the Africanized drones. The drone progeny of the F1s was used to construct a physical map. The study identified one clear QTL for defensive behavior and several candidate QTLs. This study also found several QTLs for body and wing size, as these also differ between European and Africanized bees.

HYGIENIC BEHAVIOR

Hygienic colonies are those that remove a significant number of dead, diseased, or parasitized larvae from their cells and from the nest. Hygienic bees are the individuals that perform these tasks. There is evidence that the acts of detection, uncapping, and removal of diseased larvae are separate tasks, often performed by different bees (Spivak, 1996, Spivak and Gilliam, 1998b, Arathi et al., 2000, 2006). There is a long history associated with the study of this trait, both in the context of its variation in natural colonies and in the study of its genetic basis.

Rothenbuhler spent decades working on this trait and built up an impressive body of work on many aspects of hygienic behavior (Rothenbuhler and Thompson, 1956, Rothenbuhler, 1964, Rothenbuhler et al., 1968). His most famous result was associated with the genetic basis of the trait. Rothenbuhler's basic approach was to cross colonies exhibiting high and low levels of hygienic behavior (produced with artificial selection) and then cross the F1 generation back to the hygienic line. He then classified the resulting progeny into four discrete classes, which is what one might expect if the trait is caused by two genes with Mendelian effects. He hypothesized that one locus controlled uncapping of cells containing infected larvae and another the removal of the larvae from the nest. This result became famous as an example of a complex behavioral trait with a relatively simple genetic basis.

A close look at Rothenbuhler's data, however, suggests that four discrete classes are not well supported (Rothenbuhler, 1964). Many familiar with these data were thus not surprised that later work did not support the two-gene model (Moritz, 1988, Lapidge et al., 2002). It should be stressed, however, that Rothenbuhler did many studies on hygienic bees and the overall quality of this work is excellent. There is still much to learn from reading it. In particular, his work looked at hygienic behavior in the context of removal of larvae infected with pathogens like American foulbrood, while contemporary studies use freeze-killed brood (using liquid nitrogen). Although it has been hypothesized that a common olfactory basis underlies the removal of both types of larvae (Spivak and Reuter, 2001b, Boecking and Spivak, 1999), this could turn out to be only partially true (Tsuruda et al., 2012). It may be worthwhile at some point to return to studying larvae infected with actual honey bee diseases. If this is done, a careful reading of the pioneering work of Rothenbuhler would be necessary.

Modern attempts at elucidating the genetic basis of hygienic behavior have used the QTL approach. Lapidge et al. (2002), for example, repeated Rothenbuhler's crossing scheme (crossing the divergent lines and backcrossing to the hygienic line). They made use of 358 markers (RAPDs and microsatellites) organized into 25 linkage groups and scored hygienic behavior at the colony level using now standard practices. They found six suggestive and one strongly associated QTL (Lapidge et al., 2002). Given these results, it is likely that hygienic behavior is a trait much like other quantitative traits, in that it is controlled by many genes. Oxley et al. (2010) later identified six QTLs using a different genetic map, making comparison of the two studies difficult.

Another study conducted high-resolution QTL mapping of *Varroa* sensitive hygienic (VSH) behavior using the same basic crossing and backcrossing strategy from the previous studies (Tsuruda et al., 2012). VSH bees are those that have been selected for high levels of removal of mite-infested bees. They are better at this task than are hygienic lines selected for the removal of freeze-killed brood. Going into the study, it was thought that there would be both overlapping and nonoverlapping aspects of the genetic basis of the two forms of hygienic behavior. A total of 1340 single nucleotide polymorphisms (SNPs) were used to construct a high-resolution genetic map, a great improvement on past QTL studies. One relatively clear QTL was found that explained 6% of the variance in VSH behavior. The QTL is in a relatively large segment of DNA containing 61 candidates, based on the genomic annotation. One other suggestive QTL was found.

Overall, the Tsuruda et al. (2012) study identified little of the genetic basis of VSH, and there was no overlap with the QTLs identified in the previous study on hygienic bees. Given this, it is possible that these two forms of hygienic behavior have distinct genetic bases. However, it is also possible that the high resolution of the markers counterintuitively made identification of the QTLs more difficult. If we assume that the QTLs found in previous studies (with few markers) are actually the result of variation in several genes of small effect in the linkage groups, then more resolution would make it harder to identify QTLs, as they would no longer be in large groups with cumulative effects.

GWAS Studies

Although in honey bees most statistical genetics work has used QTL mapping, it is probably the case that genome-wide association mapping (GWAS) is currently the more popular approach in general for identifying genes (or genetic variants) associated with traits (Korte and Farlow, 2013). In these methods, a large number of neutral markers are tested for an association with a trait in a single generation, making the logistics of these studies easier. Of course, the potential for confounding variables (other factors leading to associations between markers and traits) is an issue. To date, there have been few GWAS studies in bees. One recent GWAS study, however, explored the genetic basis of *Varroa* sensitive hygienic behavior (Spotter et al., 2016). A 44K SNP array was used in conjunction with behavioral observations identifying 122 hygienic bees and 122 controls. Six SNPs with high confidence association with hygienic behavior were identified. Genes near these SNPs showed promise for being candidates for hygienic behavior given their predicted functions.

Recombination

QTL studies suggested that the recombination rates in honey bees, particularly around the sex locus, are exceptionally high (Hunt and Page, 1995, Beye et al., 1999). Follow-up work showed that, genome-wide, honey bee recombination rates are higher than for any other metazoan, about 20 cm/Mb on average versus about 1.5 cm/Mb for other metazoans (Beye et al., 2006). It was later shown that high recombination rates are typical for eusocial species and are not a consequence of haplodiploidy (Sirvio et al., 2006, 2011, Stolle et al., 2011, Rueppell et al., 2016).

Beye et al. (2006) explored genomic characteristics associated with high levels of recombination. They found that regions of high GC content have high recombination rates, which was confirmed by several later studies (Liu et al., 2015, Ross et al., 2015). The frequency of simple repeats and gene size also showed a relationship with recombination rate (Beye et al., 2006). Later, Kent et al. (2012) showed that gene conversion (the biased production of GC mutations over AT mutations generated by the mechanics of recombination and DNA repair) causes the association between regions of GC bias and high recombination rates in the bee genome (figure 5.2).

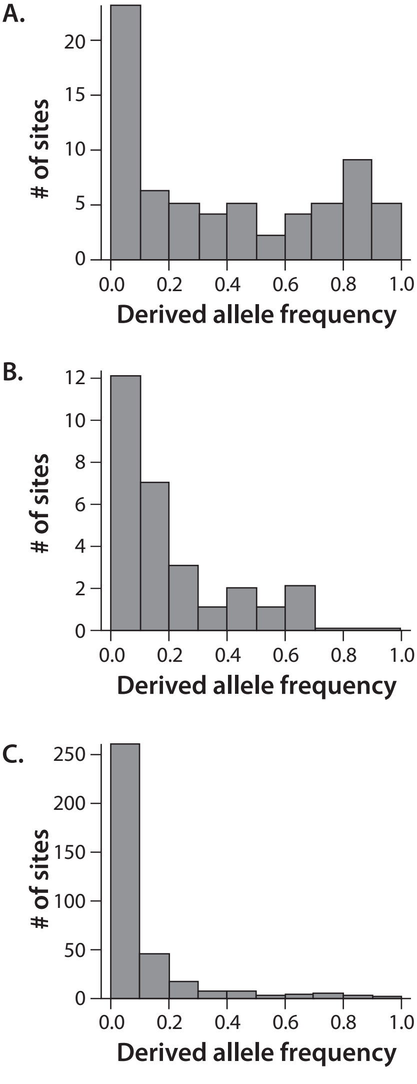


FIGURE 5.2. Frequency of genomic mutations that (A) increase GC content, (B) have no effect on GC content, or (C) decrease GC content (*from Kent et al., 2012*).

It is not clear if the high recombination rate of the honey bee, and social insects in general, is adaptive

(DeLory et al., 2020). Most work has tested two adaptive purposes for a high recombination rate related to sociality. First, it is thought that a high recombination rate, which can create new combinations of alleles, may increase colonial diversity in order to improve task allocation (Kent and Zayed, 2013). Second, it has been proposed that high genetic diversity may be a benefit for immunity, which is a pressing concern for social insects given their large population sizes and crowding within the nest (Sherman et al., 1988, Fischer and Schmid-Hempel, 2005). Most work has focused on the task allocation idea.

In support of the idea that high recombination is associated with task allocation is an observed association between high rates of recombination and genes with worker-biased expression (Kent et al., 2012, Liu et al., 2015). However, another study found that germ line genes that are methylated have low crossing rates (Wallberg et al., 2015), and when this effect is controlled for, there is no association between socially relevant genes and the recombination rate. Rueppell et al. (2012) further showed that, in principle, high recombination rates would have only a modest effect on colony genetic variation. Finally, a study exploring recombination rates in both *Apis mellifera* and the solitary bee, *Megachile rotundata*, found that the worker-biased genes linked to high recombination in *Apis* are also linked to high recombination in *Megachile* (Jones et al., 2019). This suggests that some functional aspect of these genes not associated with sociality may be driving the relationship with high recombination rates.

The Honey Bee Genome

The honey bee was one of the first species whose genome was sequenced (Weinstock et al., 2006, Elsik et al., 2014, Wallberg et al., 2019). The publication of the genome triggered a large number of studies in functional and comparative genomics. Most of this work is covered in chapter 9 on evolution. Here we review the basic attributes of the genome.

Bees have 16 chromosomes and a genome size of about 236 MB. This is comparable in size to the other holometabolous insects that were sequenced in the early phase of animal genomics. Now it is clear that many insects, particularly non-holometabolous insects, have genomes the same size or larger than is found for mammals, meaning the honey bee has a relatively small genome size. The initial genome build also documented that bees have a genome that is AT rich with quite variable patterns of GC bias. This has now been explored, as we reviewed in the preceding section.

With respect to the official gene set, it was initially only about 10,000 genes (Weinstock et al., 2006). Further work using next-gen sequencing, at the genomic and transcriptomic levels, revised this to about 15,000 genes, which is comparable to that for other insects (Elsik et al., 2014). Undoubtedly, there are many more genes to be identified. First, many genes are tissue specific and/or taxonomically restricted, and these are often missing from official gene sets (B. R. Johnson et al., 2013, Jasper et al., 2015). Second, many classes of functional RNA are poorly annotated, and still others are poorly understood and/or yet to even be discovered (Cech and Steitz, 2014, Kung et al., 2013, Lee, 2012, van Bakel et al., 2010). Long intergenic noncoding RNAs (LincRNAs), in particular, may turn out to be much more common than previously thought, as they are difficult to find using methods based on homology but likely play many roles in the cell (Batista and Chang, 2013, Nagano and Fraser, 2011, Tsai et al., 2010). In general, the annotation for even a well-assembled genome like the honey bee is a work in progress, and it is good to keep in mind that major additions to the official gene set are likely to come. For example, Wallberg et al. (2019) greatly improved the honey bee genome using multiple long-read next-gen sequencing technologies.

Most of the interest in the genome, of course, centers on gene content. It has been shown that bees have distinctive and functionally important gene set attributes. For example, bees have smaller numbers of genes in immune and detoxification pathways relative to most other insects (Evans et al., 2006, Claudianos et al., 2006). Later work has found that these are taxonomic characteristics and are not associated with the evolution of sociality (Barribeau et al., 2015, Berenbaum and Johnson, 2015). Bees have also been shown to have a functional methylation gene set (Wang et al., 2006, Foret et al., 2012). It is now known that most invertebrates do, but the honey bee was the first insect for which these genes were identified in full (*Drosophila* is missing this important mechanism for gene regulation). Bees also have a large number of odorant receptors relative to solitary insects, like flies (Robertson and Wanner, 2006, Weinstock et al., 2006). It has since been shown that most social insects, particularly ants, share this trait (Leal, 2013, Smith et al., 2011a, 2011b). Expansion in several gene families, such as the royal jelly proteins, have also been identified (Drapeau et al., 2006, Buttstedt et al., 2014).

6

Neurobiology

Much of neurobiology is concerned with (1) mapping out complex neuronal structures, (2) exploring the function of individual neurons, or (3) elucidating how larger neuronal circuits process information to produce adaptive behavior. In this chapter, with respect to work on honey bees, we give a concise review of (1), omitting technical discussion relating to technique, and focus mainly on (3). With respect to (2), detailed discussion of cell physiology, independent of particular context, is quite complex and not at all bee centric. We thus emphasize work on topics such as color vision, olfaction, learning and memory, timekeeping, and so forth, as these have been the subject of much work in bees. We also cover neurogenomics, a rapidly growing field. Finally, neurobiology is a subject that requires much background knowledge to grasp even basic results. This chapter will thus be less accessible to those without a background in the subject than is the case for any other chapter.

The Nervous System

The insect nervous system is traditionally broken up into three components: the central nervous system (CNS), the visceral nervous system, and the peripheral nervous system (PNS). We discuss each in turn.

The Central Nervous System

The insect CNS is composed of the brain and the string of ganglia descending down the bee's body in a ventral nerve cord. The honey bee brain is about a cubic millimeter in volume and has about a million neurons (Menzel and Giurfa, 2001). It is thought to be the result of the fusion of three ancestral

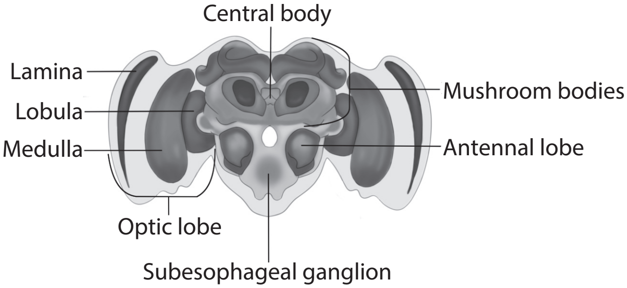


FIGURE 6.1. The honey bee brain with major neuropils (*redrawn from Ribi et al., 2008*).

ganglia (Strausfeld, 2012). A drawing of the brain and its most prominent components is shown in figure 6.1. The brain is classically broken up into three sections: the protocerebrum, the deutocerebrum, and the tritocerebrum (Brandt et al., 2005). We discuss brain anatomy in detail later in conjunction with the relevant sensory systems and processes. Here we simply point out prominent neuropils. The protocerebrum contains the large optic lobes associated with visual processing, the central complex associated with higher-order processing of information from many sources, and the mushroom bodies (MBs), which are also associated with learning and memory (Homberg et al., 2011). The pars intercerebralis is also here and houses neurosecretory cells that send hormones to the corpora cardiaca that integrate the nervous and endocrine systems. The deutocerebrum contains the antennal lobes (ALs), where olfactory and mechanosensory processing occurs, along with what used to be called the dorsal lobe, now known as the antennal mechanosensory and motor center (AMMC). In the manner the insect brain is typically drawn, the AMMC sits below the AL. The tritocerebrum is a bridge between the brain, the visceral nervous system, and the ganglia of the ventral nerve cord and is quite small in bees.

The fused suboesophageal ganglion (SEG), sometimes called the suboesophageal zone, sits behind the brain (Ito et al., 2014). It is thought to be the result of the fusion of three ancestral ganglia associated with the mandibles, the maxillary palps, and the labia (Strausfeld, 2012). The SEG controls these oral appendages, along with the salivary glands, and other structures in the neck area. It is also where first-order gustatory processing occurs. The next posterior ganglion is the prothoracic ganglion, which controls the forelegs. The large pterothoracic ganglion is next. This structure is also thought to have resulted from the fusion of ancestral ganglia (the last two thoracic ganglia and the first abdominal ganglion). This neuropil controls the wings, mid and hind legs, flight muscles, and the activities of the first abdominal segment. The metasoma (abdomen) contains five segmental ganglia. The first four are simple (not thought to be fusions of ancestral ganglia) and control either activities in their own or a neighboring segment. The final ganglion, the caudal ganglion, is also a large ganglion thought to have resulted from fusions of smaller ganglia. This structure controls the genitalia, the many glands associated with them, and the sting apparatus. It also integrates with the visceral nervous system.

The Visceral Nervous System

The visceral nervous system is analogous to the vertebrate autonomous nervous system and controls functions associated with digestion, excretion, and respiration (Boleli et al., 1998). It is composed of a structure called the frontal ganglion that controls the foregut and the crop. This ganglion communicates with the hypocerebral ganglion that innervates the gut and with the corpora cardiaca and corporal allata (Snodgrass, 1956). The hindgut is innervated by the caudal ganglion. The visceral nervous system is thus connected to the brain, the endocrine system, and the segmental ganglia.

The Peripheral Nervous System

The peripheral nervous system is the umbrella term for all the neuronal tissue and associated structures located outside the CNS. This includes the diverse sensory structures and their associated sensory neurons that project axons to the CNS, as well as the motor neurons projecting from the CNS to various effectors (muscles, glands, etc.) throughout the body.

Sensory Systems: From Structures to Neural Processing

Insect sensory structures are composed of two cell types: sensory neurons that detect and transduce environmental stimuli into patterns of nerve firing, and accessory cells that aid in this task, often by the construction of a structure that facilitates transduction. The different sense organs have common properties, but also much that is unique, as can be expected when considering the diversity of environmental stimuli that must be transduced.

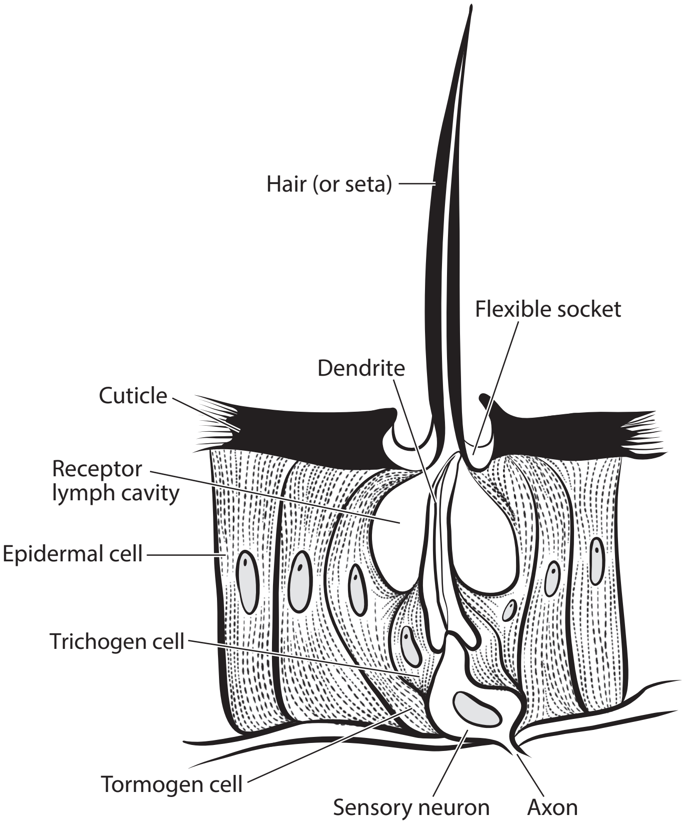

Hair (or seta)
Flexible socket
Dendrite
Cuticle
Receptor lymph cavity
Epidermal cell
Trichogen cell
Tormogen cell
Sensory neuron
Axon

FIGURE 6.2. A generalized trichoid sensilla (*redrawn from Chapman, 1991*).

Mechanical Senses

Some of the simplest insect sense organs respond to deflection, either from contact with a solid surface or from the wind. The most basic of these, common to all insects, are the trichoid sensilla shown in figure 6.2 (Chapman, 1991). This structure is composed of a hair suspended by a thin membrane into a pit in the cuticle such that deflection of the hair tugs the dendrite (Keil, 2012). Transduction happens via mechanical stress on the dendrite, which opens ion channels (Gillespie and Walker, 2001). Trichoid sensilla are used in many contexts. They are on the cuticle's surface, for example, where they function as touch receptors. These same sensilla respond to gusts of air. Trichoid sensilla often have pores that allow them to function as airborne or contact chemoreceptors, mechanoreceptors, or both.

Trichoid sensilla also occur at most joints. Here flexion of the appendage excites the dendrite. In most cases, degree of deflection determines the rate of firing. These sensors are thus useful for basic proprioception, as insects typically lack the muscle and tendon stretch receptors of vertebrates. A particularly derived patch of trichoid sensilla (such patches are often called plate organs) sits behind the head and is involved in visual guidance and gravity sensing, as it records the position of the head in relation to the thorax. This organ is important for the waggle dance as it underlies the ability to transform an angle in reference to the sun to an angle in reference to gravity (Brockmann and Robinson, 2007).

A more derived form of sensilla, the campaniform sensillum, also functions as a proprioceptor, as it senses stress on the cuticle. Its design is similar to the trichoid sensilla except that the dendrite articulates with a dome-like structure more or less level with the surface of the cuticle. Compression along the cuticle deforms the dome, triggering firing of the sensory neuron attached to it. A yet more derived and sensitive class of proprioceptors are called scolopodia (Hallberg and Hansson, 1999). These are internal and typically connect at both ends to the inner cuticle, often in small structures or at curved surfaces. Here many sensory neurons are connected to a plate embedded in the cuticle. Stress on the plate is measured by the firing of the sensory neurons.

It is the scolopodia that evolution elaborated upon for vibration and sound reception in insects. The honey bee has several of these structures, called chordotonal organs, of which two have received experimental attention (Field and Matheson, 1998, Yack, 2004). We start with the subgenual organ, which is located in the tibia and senses vibrations of the substrate on which the bee is standing (Kilpinen and Storm, 1997, Storm and Kilpinen, 1998). This organ has about 80 scolopodia. The subgenual organ is not associated with a joint, but rather jostles in the fluid-filled space such that when the legs vibrate up and down the sensory neurons fire at a corresponding rate. The second chordotonal organ, which has received more experimental attention, is the Johnston's organ (JO), located in the second segment of the antenna, called the pedicel (Ai et al., 2009, Dreller and Kirchner, 1993, Kirchner, 1994). This structure is similar in design to the subgenual organ but more complex, having 240 scolopodia. Each scolopodium is associated with a few sensory neurons. The JO is used for a variety of functions in different insect species. In flight, for example, the antenna is deflected by resistance from the air to a degree that correlates with speed, and this is recorded by the JO (Ai, 2013).

With respect to sound, it was long thought that bees could not hear, as they lack a tympanic organ (an insect eardrum). However, this would only rule out the sensing of pressure waves, not velocity of particle movement. With respect to near-field sound (net movement of air particles near a sound source), experiments have shown that the JO is responsive to this form of sound (Dreller and Kirchner, 1993, Kirchner, 1994). Waggle-dancing bees produce bursts of this form of sound (air flow) as they dance, which are sensed by the JO of the bee following the dance.

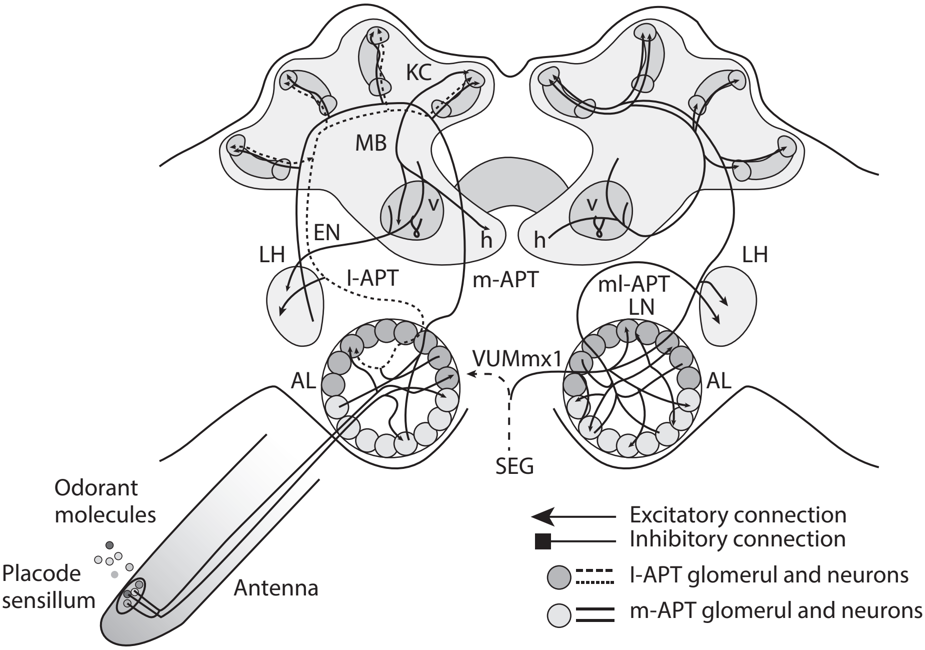
AUDITORY PROCESSING IN THE BRAIN

Neurons from the JO project via the antennal nerve to two processing centers, the antennal mechanosensory center (AMMC) and the superior posterior slope (SPS) (Ai and Itoh, 2005, Ai et al., 2007). The SPS may be involved in the integration of visual information with auditory signals from the JO. The AMMC is thought to be involved in integration of mechanosensory signals from sensilla at the base of the antenna (and elsewhere) with auditory information from the JO and may be involved in some of the information processing underlying the waggle dance. With respect to mechanism, several interneurons in the AMMC with dense arborizations have been shown to be responsive to spikes of experimental acoustic simulation via the JO (Ai et al., 2009).

Chemical Senses

Olfaction

Figure 6.3 provides a map of the olfactory system in bees (Sandoz, 2012). Honey bees smell with their antennae. About 3000 pore plates are found here (this varies with sex and caste), each innervated by a variable number of sensory neurons (up to 35) called olfactory receptor neurons (ORNs) (Kelber et al., 2006). ORN dendrites sit in a fluid-filled space near the opening to the pores, and based on work in the fruit fly, odorants, which are typically hydrophobic volatile compounds, are carried to them by odorant-binding proteins that solubilize them (de Bruyne and Baker, 2008). ORNs express odorant receptors (ORs). These are highly derived G protein coupled receptors (GPCRs) that bind to the odorant with their extracellular domain, and through their intracellular domain cause an ion channel to open (Sato et al., 2008, Wicher et al., 2008). They are not closely related to the odorant receptors in vertebrates (Leal, 2013). Two kinds of ORs work together to transduce olfactory stimuli. One is called *orco*, which is expressed in every ORN and does not bind odorants


KC
MB
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EN
LH
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l-APT
m-APT
ml-APT
LN
VUMmx1
AL
AL
SEG
Odorant molecules
Placode sensillum
Antenna
Excitatory connection
Inhibitory connection
l-APT glomerul and neurons
m-APT glomerul and neurons

FIGURE 6.3. Honey bee olfactory system (*from Sandoz, 2012*).

(Stengl and Funk, 2013). *Orco*, often called an obligate coreceptor, is part of a protein complex with another type of OR that binds odorants. This other OR determines the odorant response properties of the ORN. The term OR from here out is restricted to mean these odorant-binding ORs. The honey bee has about 170 different ORs (Robertson and Wanner, 2006, Karpe et al., 2016). Each ORN generally expresses a single OR type, and each OR type differs in its tuning breadth, that is, it differs in the number of odorants it can respond to (Kreher et al., 2005). The ORs responsive to pheromones (as opposed to what are sometimes called general odors) are likely the most finely tuned, as a single OR type can respond to single odorants, as has been shown for queen pheromone (Wanner et al., 2007, Sandoz et al., 2007).

Tens of thousands of ORNs project to the antennal lobe (AL) (Matsumoto and Hildebrand, 1981). In general, the function of the AL is to discriminate the odors that the antenna has intercepted (Galizia and Menzel, 2001). This begins with two neural mechanisms: gain control and contrast enhancement (Sandoz, 2012, Szyszka and Galizia, 2015). Gain control refers to amplifying or suppressing receptor neuron inputs. Weak signals are thus amplified, and strong signals are decreased in amplitude. Contrast enhancement refers to clarifying, refining, or translating the raw signal. In general, every ORN expressing the same OR projects to the same functional structure in the AL, called a glomerulus (Vosshall et al., 1999, Galizia et al., 1999a). Here the ORNs, which form an outer ring, make synapses with two other types of neurons—local neurons (LNs) and projection neurons (PNs)—in the center of each glomerulus (Flanagan and Mercer, 1989, Fonta et al., 1993). The 4000 LNs can be broken into five categories based on electrophysiological responses to odorants and the neurotransmitters they use (Sandoz, 2011, Meyer et al., 2013). The honey bee has about 165 glomeruli, a number that corresponds to the number of ORs (Galizia et al., 1999b, Robertson and Wanner, 2006).

The PNs project, via several tracts (allowing for parallel processing of olfactory information), from the glomeruli to higher brain centers for further processing and integration with other sensory systems (Abel et al., 2001, Kirschner et al., 2006, Carcaud et al., 2012, Roessler and Brill, 2013, Zwaka et al., 2016). Most of the 800 PNs project from single glomeruli, but a minority extend from several (Hammer, 1997, Rybak, 2012). The two primary targets of PNs are the mushroom bodies (MBs) and the lateral horn (LH), which may be the site of processing for stereotyped, innate olfactory behaviors, such as that related to mating and foraging (Strausfeld, 2002, 2012). The mushroom bodies have received considerably more attention than the LH. Here Kenyon cells (KCs), the intrinsic interneurons of the MB, are innervated by PNs from the AL (along with those from the other senses). Honey bees have about 360,000 KCs (split between two hemispheres), which is similar to what is found in other social Hymenoptera, but far higher than what is found in insects of many other orders (Alten, 1910). Many studies have shown in honey bees that the organization of the MBs changes with the transition to foraging, and that this may be associated with the learning and memory of new stimuli (Withers et al., 1993, 1995, Fahrbach et al., 1995, 2003, Maleszka et al., 2009).

SPATIAL CODING OF ODOR IN THE AL

Joerges et al. (1997) confirmed, using calcium imaging, the long-standing idea that odors evoke distinct spatiotemporal patterns of activation across glomeruli in the AL. Also confirmed was that mixtures are coded by both additive and nonadditive mechanisms. Presumably, the additive effects are due to activity in the ORNs, while the nonadditive effects stem from the inhibitory activity of LNs. Sachse et al. (1999), again using calcium imaging, then presented bees with a large series of odors that varied in chain length and functional

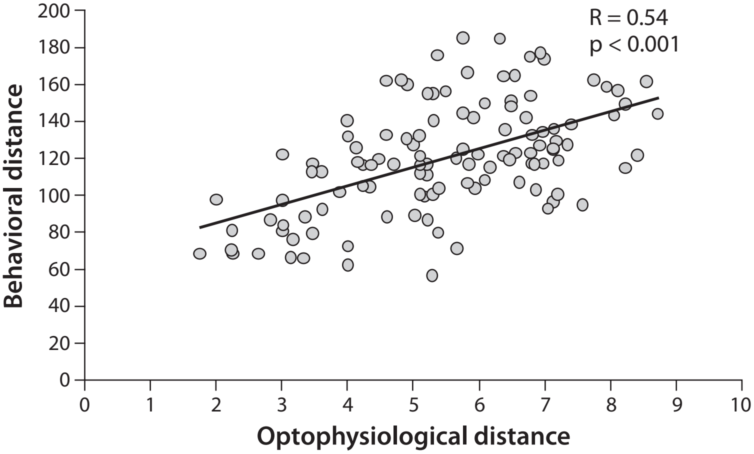


FIGURE 6.4. Correspondence between optophysiological similarity measured using calcium imaging and behavioral measure of olfactory similarity using PER (*from Guerrieri et al., 2005*).

group to better clarify AL coding. The results were quite expansive and detailed, but some general conclusions were drawn. For example, glomeruli that were sensitive to particular functional groups were often broadly tuned with respect to the length of the carbon chain. Overall, there was much variation in the degree of total activation across the AL with respect to the response to particular functional groups. Work then focused on addressing whether odors evoke similar responses across glomeruli across individuals. In other words, does the same glomerulus across individuals respond to a particular odor? The answer appears to be yes, as demonstrated by Galizia et al. (1999b). Finally, it was shown that these spatial patterns match well with behavioral data on generalization (Guerrieri et al., 2005). That is, the correlations between the spatial patterns for odors correlates with the likelihood that one odor will be perceived as similar (show a generalization effect) to another in the proboscis extension response (PER) protocol (figure 6.4), which is described in depth in chapter 7.

OLFACTORY LEARNING

The reward component to associative learning is implemented by the innervation and response properties of an identified neuron, the ventral unpaired median maxillary neuron or VUM-mx1, projecting from the SEG (Hammer, 1993, 1997). We explore this further in the section on gustation that follows.

In this section, we focus on the spatiotemporal patterns that result during associative learning of odors and then on the responses associated with nonassociative learning. PER is an olfactory conditioning protocol that allows one to train bees to associate a given odor with a reward. Faber et al. (1999) conducted in vivo calcium imaging of the AL during the PER process. The spatiotemporal pattern of activation across the glomeruli during differential training with conditioned stimuli (CS), rewarding one odor and not a second, was found to change for a CS+ (the rewarded odor) but not for a CS– (the unrewarded odor). The consequence was a decoupling of whatever initial correlation existed between the spatiotemporal patterns for the two odors (thus moving them apart in the olfactory space). The response to the CS+ also became stronger. Later work has confirmed and expanded upon this result without changing the take-home message (Sandoz et al., 2003, Fernandez et al., 2009, Rath et al., 2011). Rath et al. (2011), for example, found that inhibitory feedback from the LNs is likely the cause of the change in the response to the CS+. A more recent study by Locatelli et al. (2016) showed that the same process of decoupling of the spatiotemporal pattern in the AL occurs when complex floral-like blends are used instead of simple odors. Classic Hebbian reinforcement (use of a circuit strengthens its connections) is thought to underlie these results, although a clear demonstration at the synaptic level is lacking.

Another body of work has explored nonassociative learning in the AL using the same methods. Nonassociative learning, such as habituation, is important because it allows the animal to ignore common odors of no informative value. Locatelli et al. (2013) showed that changes in the activity of the AL occur when odors are presented repeatedly without a reward. Their approach was to present a bee with the same odor 40 times, then present a second odor in conjunction or separate from the first. They found that when the first and second odors were presented independently, the spatiotemporal patterns in the AL to either odor was not changed. When the second odor was presented with the first odor (already experienced many times), however, a novel spatiotemporal pattern more like the second odor was recorded. The authors interpreted this as meaning that the olfactory system places emphasis on recognizing novel odors, relative to those commonly encountered.

LEARNING IN THE MBS

We first review MB structure in more detail before exploring learning (figure 6.3). The MB calyx is composed of the lip, the collar, and the basal ring (Gronenberg, 2001). Olfactory input arrives in the lip and the basal ring (this area also receives visual input). Here boutons, or swellings of the PNs, mediate the connection to the KC dendrites (Paoli and Galizia, 2021). These boutons are also innervated by extrinsic feedback neurons from the MB itself (covered shortly) and neurons carrying modulatory information from other sensory systems. These interfaces, called microglomeruli (MGs), are therefore the sites of much information processing and have received considerable study (Groh and Roessler, 2020). The KC axons project down to the pedunculus and lobes, where they synapse with other classes of MB neurons. Chief among these are feedback neurons of two sorts and output neurons that project to the LH and other brain centers (Rybak and Menzel, 1993). The feedback neurons either are local and mediate KC axonal activity in the pedunculus, or project back to the MGs to create a feedback loop (Zwaka et al., 2018). The cells that create the feedback loop, called A3 neurons, have also received attention as they clearly modulate olfactory coding.

As for the AL, our understanding of MB function derives mainly from calcium imaging and recording studies in which key neurons are stimulated or neurotransmitters are uncaged. Faber and Menzel (2001) followed up on their AL work, previously reviewed, with a similar study in the lip region of the MBs. The results were quite similar to the AL work, in that activity was stronger for the CS+ than the CS− after differential PER conditioning. Szyszka et al. (2005) continued this work with more expansive imaging studies of the behavior of AL PNs, their boutons, and the KCs during odor exposure. They found that KC responses to odors were sharper and shorter than responses in the AL PNs (figure 6.5). This likely reflects a filtering of the information to a sparser, more compact form. KCs were also quite specific in their response to odors. A follow-up study found that repeated exposure to an odor decreased the response of KCs, but when an odor was paired with the unconditioned stimulus (and hence learned) this decrease diminished (Szyszka et al., 2008). These studies looked at class II KCs. Finally, Hourcade et al. (2010) showed that PER conditioning leads to an increased number of MGs in the lip area of the MB. Thus, changes to the behavior of intact circuits, and the formation of new circuits in the MB, are likely associated with olfactory learning.

Another body of work has looked at the extrinsic neurons that create feedback from the KCs to the MGs. These neurons have been shown to modulate their activity in response to odor presentation (Grunewald, 1999a, 1999b). As for several other neuropils, repeated exposure without reward decreases

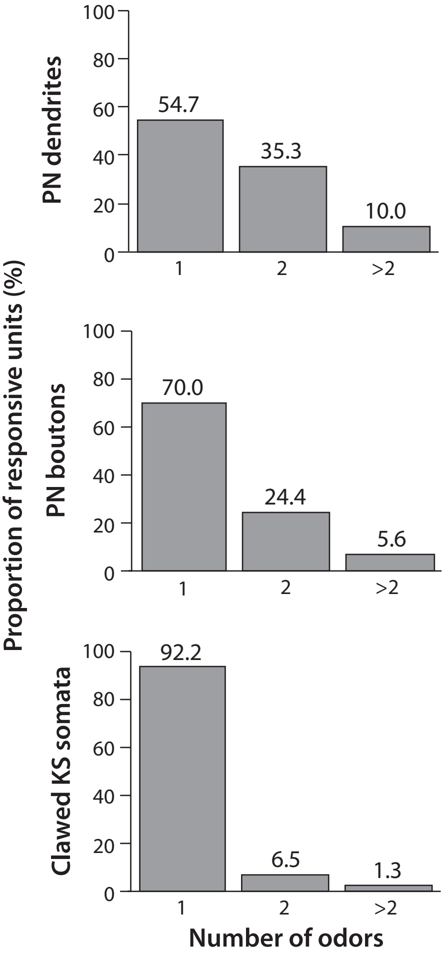


FIGURE 6.5. Decreasing percentage of responsive units as a signal moves up the olfactory system. A high percentage of PNs in the AL respond to multiple odors, while very few do in the MBs (*from Szyszka et al., 2005*).

their activity, while learning with repeated exposure prevents the decline (Haehnel and Menzel, 2010, 2012). There is thus overlap in the response to odor, and in learning, in several populations of neurons in this large neural network (encompassing the whole MB), making it difficult to determine which population of cells is responsible for what function.

MEMORY-FOCUSED STUDIES

The classic models for what memories are at the neurobiological level focus on the formation of new or stronger connections between neurons (Burgess et al., 2002, Lisman et al., 2002, Deng et al., 2010). This can result from new synapse formation or, probably more commonly, from the strengthening of existing connections between neurons (Squire, 1986). Work on honey bee memory has elucidated aspects that are analogous, and perhaps homologous, to those revealed from work on phylogenetically distant model systems. Early work used a combination of behavioral studies and cold anesthesia to show that bees have short-, mid-, and long-term memory (STM, MTM, and LTM, respectively) located in both the ALs and the MBs (Menzel et al., 1974, Menzel and Muller, 1996, Menzel, 1999). Although time ranges for these memory stages were stressed in the early literature, it is likely that these are context specific (Paoli and Galizia, 2021). It is easier to state that STM is short lived and based on posttranslational modifications (PTMs) and electrical activity. It is nonspecific, probably associated with a general arousal, and quickly fading. MTM is associated with translation, while LTM is associated with transcription and translation. It was previously thought that STM and MTM could be induced with a single PER trial, while LTM required three trials; however, Villar et al. (2020) showed that even a single trial can lead to LTM formation.

The molecular basis of memory formation is based on multiple processes that operate in parallel. First, in bees, VUM-mx1 releases octopamine (OA) into both the AL and the MBs, priming them for learning the odor (Hammer, 1993, 1997, Hammer and Menzel, 1998). In the MBs, coincident activation, which is further a conserved aspect of LTM across the tree of life, is thought to be mediated by the NMDA-type glutamate receptor (Bollen et al., 2014). This ionotropic receptor requires both release of glutamate from the presynaptic neuron and depolarization of the postsynaptic neuron for the channel to open (Menzel and Manz, 2005). Signals from the AL PNs, in conjunction with other partners, thus open the pore in the NMDA receptor channel, allowing a rise in intracellular Ca^{2+}, an important second messenger for multiple signal transduction pathways (Muller, 1996, Schwaerzel and Mueller, 2006). Elevated Ca^{2+} activates protein kinase A (PKA) and CREB, via elevated cAMP, along with activation of protein kinase C (PKC) (Grunbaum and Muller, 1998, Muller, 2000, Eisenhardt et al., 2003, Jarome and Helmstetter, 2013, Eisenhardt, 2014, Matsumoto et al., 2014). In support of this, release of glutamate in the MB after odor presentation leads to LTM (Locatelli et al., 2005), and knockdown of the NMDA-type receptor interferes with LTM (Mussig et al., 2010). Knockdown of PKA interferes with LTM (though not STM) (Fiala et al., 1999). Activation of PKA and PKC ultimately leads to changes in transcription and translation to change connectivity at the synapse, although the downstream effects still await serious attention. It has been shown that epigenetic mechanisms are involved (Biergans et al., 2016).

Gustation

Gustatory structures are located on the antenna, the mouthparts, the oral cavity, and on the forelegs (Goodman, 2003). Most work has focused on the antenna, but studies have been conducted on all parts other than the oral cavity. The sensilla themselves are either hair- or peg-like with a single pore at the tip through which tastants enter a fluid matrix (Esslen and Kaissling, 1976). The sensory neurons, called gustatory receptor neurons (GRNs), project their dendrites near the pore where they encounter tastants. There are between three to six sensory neurons associated with each sensillum, depending on the location (Mitchell et al., 1999). One of the sensilla is often a mechanoreceptor used to "feel" the food in addition to taste it (Whitehead and Larsen, 1976b). Sensilla are typically specialized (for sweet, salty, or amino acids), and how many neurons are devoted to each taste varies across the bee's body (Whitehead and Larsen, 1976a, Haupt, 2004, de Brito Sanchez et al., 2005, Lim et al., 2019). Sensilla on the antenna, mouthparts, and the tarsi, for example, are particularly sensitive to sugars. Other sensilla on the tarsomeres are salt receptors (de Brito Sanchez et al., 2014).

GRs are highly derived G protein coupled receptors distantly related to ORs (Bestea et al., 2021). They are ion gated channels that open upon tastant binding to trigger action potentials (Sato et al., 2011). Individual GRs are responsive to sugars, salts, amino acids, and water (de Brito Sanchez, 2011). Bees may only have GRs sensitive to bitter compounds on the mouthparts, and they do not seem relevant for many important gustatory behaviors (de Brito Sanchez et al., 2005, 2015, Montell, 2009, Wright et al., 2010). Harnessed bees, for example, drink freely from sugar water laced with bitter compounds. However, this induces a general malaise, which can then form the basis for an aversive memory, thus allowing for some sensitivity to bitter compounds (Ayestaran et al., 2010).

GRs project to the SEG, a structure for which gustatory processing is a major function (Mitchell et al., 1999). Most work on processing of gustatory information has focused on an identified neuron, the VUM-mx1, which is octopaminergic. This neuron is activated upon sucrose presentation, based on electrical recordings (Hammer, 1993, 1997). Further, stimulation of this neuron during olfactory conditioning can trigger memory formation, mimicking a sucrose reward (Hammer and Menzel, 1998). Likewise, local injection of octopamine mimics sucrose presentation, while blocking of function, by knockout of OA receptors, interferes with associative olfactory conditioning (Hammer and Menzel, 1998, Farooqui et al., 2003). Thus, input from this neuron mediates the control of the sugar reward effect on olfactory learning. Consistent with this, VUM-mx1 projects to the MBs and the LH (Schroeter et al., 2007). More than a dozen other VUMs have been described in the SEG, and they have been shown to be responsive to water and salt but have received little experimental attention (de Brito Sanchez, 2011).

The Visual System

Visual Anatomy

Optical systems focus light onto a photosensitive surface where transduction occurs via photosensitive pigments. The pigments, generally called rhodopsins, comprise a G protein coupled receptor (the protein opsin) that surrounds a form of vitamin A (Findlay and Pappin, 1986, de Ibarra et al., 2014). When the rhodopsin intercepts a photon within a range of wavelengths, the vitamin A changes configuration, which causes the GPCR to change shape and activate a signal transduction cascade within the photoreceptor cell that opens an ion channel, allowing the cell to become electrically excited. Visual sensory cells give finely graded continuous responses to light, are metabolically costly, and are among the most complex sensory structures in terms of quantity and diversity of the information they collect.

Unlike many larval insects, such as caterpillars that have simple eyes, called stemmata, the honey bee larva is blind. The adult bee, in contrast, has two types of eyes. The simpler of the two are the ocelli (Ribi et al., 2011). These are three single-lens eyes on the top of the head in a triangular formation. Each lens gathers light from a relatively large angle, but the image is not focused on the retina, which has only a few photoreceptors, so ocelli are not thought to form a high-resolution image (Wilson, 1978, Rieger et al., 2003). Rather, they function as fine-scale light collectors. They are used in flight as horizon detectors and to control flight orientation (Taylor, 1981, Kastberger, 1990).

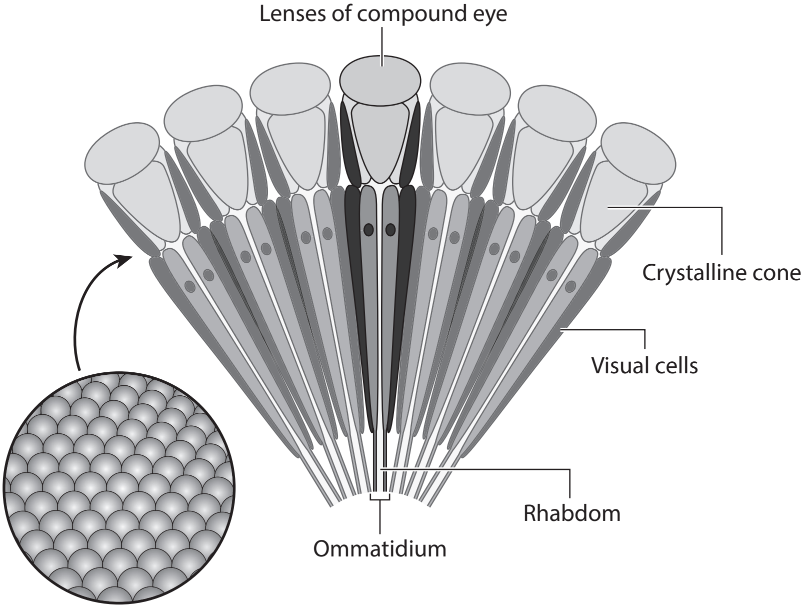


FIGURE 6.6. Structure of the insect compound eye, ommatidium, and rhabdom (*redrawn from Srinivasan, 2011a*).

The compound eyes are composed of thousands of simple eyes called ommatidia (Avargues-Weber et al., 2012). Each honey bee compound eye has about 5500 ommatidia, although as we reviewed in chapter 4, this varies with sex and caste. Figure 6.6 shows the basic structure of an insect ommatidium. A corneal lens and a crystalline cone focus light onto the rhabdom, which is formed from the fused rhabdomeres (dendrites) of the nine sensory neurons associated with each ommatidium (Menzel et al., 1989, Wakakuwa et al., 2005, Gribakin, 1972, Menzel and Blakers, 1976). Eight of the neurons have rhabdomeres that span the rhabdom while the ninth has a shorter rhabdomere that sits at the base of the rhabdom. The photoreceptors themselves are located in microvilli, which are extensions of the sensory cells. The microvilli from different rhabdomeres are twisted about the rhabdom in most of the eye, which prevents polarized light detection but improves light reception. Microvilli are not twisted, however, in the dorsal rim area (DRA) of the eye in order to detect the plane of polarization of light (Rossel et al., 1978, Rossel and Wehner, 1984, Labhart and Meyer, 2002). Photoreceptors, as mentioned before, come in three forms. These are the receptors for short (S, 344nm, UV), medium (M, 436 nm, blue), and long (L, 544, green) wavelength light (Menzel and

Backhaus, 1991, Peitsch et al., 1992). The eight walls of the ommatidium house pigment cells, which block light that would otherwise hit the rhabdom from too wide an angle. This serves to facilitate collection of light from just one distinct area, which increases the clarity of the image formed (Borst, 2009).

Each of the nine neurons associated with an ommatidium expresses a single photoreceptor type (Menzel and Blakers, 1976, Menzel et al., 1989). There are more green receptors overall because the green receptor alone is used for motion and pattern recognition (Giurfa et al., 1996b, 1997, Srinivasan, 2010). Ommatidia come in three classes, based on which photoreceptive pigments they contain. According to molecular studies, all three classes have six green receptors (Wakakuwa et al., 2005). Type 1 ommatidia have, in addition, one UV and one blue receptor. Type 2 ommatidia have two UVs and type 3 have two blues. The last neuron (each ommatidium is associated with nine), is the neuron that sits at the base of the rhabdom, which has an unknown sensitivity.

Visual Processing in the Brain

The optic lobe, shown in figure 6.1, has three major neuropils: the lamina, the medulla, and the lobula. Each neuropil retains a retinotopic organization with neighboring cartridges, or columns, of neurons processing information from neighboring ommatidia. L and M receptors project from each ommatidium to distinct visual cartridges in the lamina (Menzel, 1974, Menzel et al., 1989, Vorobyev and de Ibarra, 2012, de Ibarra et al., 2014). Each cartridge is associated with four different types of lamina monopolar cells, as the interneurons confined to this neuropil are called (Menzel, 1974). There is extensive branching of these neurons with one another and with the sensory neurons projecting from the eye to each cartridge. The lamina is involved in preliminary processing of visual information (Desouza et al., 1992). S (UV) photoreceptor axons bypass their lamina cartridge and project directly to the medulla (Dyer et al., 2011). Neurons from the lamina also project to the medulla, but the medial to lateral spatial representation of the columns of neurons is reversed here (Ribi and Scheel, 1981). The medulla is thicker than the lamina and is more structurally complex. The columns here contain many more intrinsic neurons processing information from the region of space viewed through their dedicated ommatidium. Many fibers also begin the comparison of information across columns. The medulla houses color opponent neurons (more on this below), suggesting that this is where much color processing occurs (Kien and Menzel, 1977a, 1977b, Backhaus, 1991, Hertel et al., 1987, Hertel, 1980). Neurons from the medulla project to the lobula, which is characterized by outer and inner sections. Only the outer section retains spatial information (Hertel et al., 1987), but how the lobula functions, in general, is poorly understood. However, it is clear that achromatic visual motion is represented here and color information is further processed. Neurons from both the medulla and the lobula project to the higher brain centers, including the mushroom bodies and the lateral protocerebrum of the central brain (Avargues-Weber et al., 2012). This is presumably where the visual stimuli (chromatic and achromatic) are integrated with other sources of sensory information, but further processing likely happens here as well (Menzel, 1999, Giurfa, 2007, Ehmer and Gronenberg, 2002, Mobbs, 1984).

Finally, with respect to the functional properties of the neuropils associated with vision, little is understood in concrete terms. The clearest result in bee vision (although this is true of visual systems in general) is that color, polarization, and achromatic processing happen separately and in parallel (Paulk et al., 2008, 2009a, 2009b, Dyer et al., 2011, 2008, Mota et al., 2011). Information related to either color, motion, or pattern travels along separate tracts and is processed in separate modules within the optic lobe, and in the higher brain centers (Kaiser and Liske, 1974, Srinivasan and Lehrer, 1984). Much of the highest-resolution work exploring these parallel paths of processing has taken place in bumble bees, but it is thought that there are few differences between bumble bees and honey bees in these respects.

Color Vision

The perceptual components of color have brightness, hue, and saturation. Hue and saturation relate to chromatic aspects of light, while brightness is achromatic. Hue is a function of wavelength, whereas saturation relates to the richness of the color. Generally speaking, an animal can see color if it can distinguish between lights of different spectral composition when their brightness is equal. There are two main ideas to understand with respect to color discrimination: color opponency and color spaces.

Photoreceptors of all organisms provide ambiguous information about the wavelength of light they have transduced; thus, the network of higher-order neurons must resolve this ambiguity. For instance, each of the three bee rhodopsins has a wavelength to which it is most sensitive: 344 nm, 436 nm, and 544 nm (figure 6.7). Their sensitivity curves, however, are still 50% sensitive to light 50 nm away from the peak. Thus, the so-called green photoreceptor

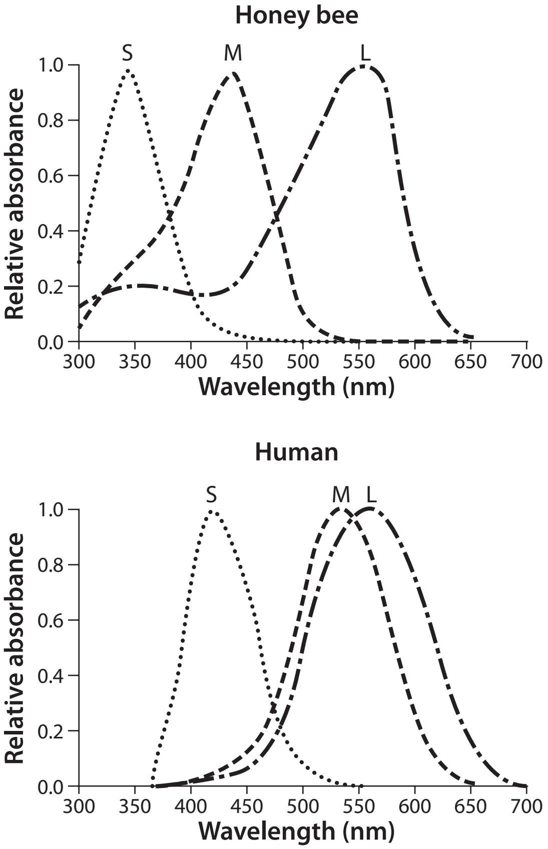


FIGURE 6.7. Honey bee color vision is shifted to the left relative to humans. The three bee pigments have peaks in the UV, blue, and green regions of the spectrum (*from Avargues-Weber et al., 2012*).

responds similarly in probability terms to a single photon of green light at 544 nm as it does to two photons of orange light at 594 nm. Because the sensitivity curves of the three spectral types of photoreceptors overlap, discrimination of the wavelength occurs through comparison of the relative excitations across spectral types that view the same region of space, and spectral discrimination is finest in the regions of overlap (von Helverson, 1972).

The principal type of comparison in all organisms is color opponency. This is the idea that the perception of hue is based on an organization of colors along axes with opponent colors at either end. The two colors are opponents in that a color cannot have the character of both at once. In the classic human example, a color cannot be both reddish and greenish, but either redder or greener. Hence, red and green form one axis. Given that they fall along one axis, it stands to reason that there must be a point at which a color is neither red nor green, or in other words appears white. Demonstrating the existence of this point by combining light of different colors is a major source of confirmation for the color opponency idea. From a neurobiology perspective, a color opponent neuron is one that shows a combinatorial response to wavelengths of light along the opponent axis. One clear example is a neuron that shows an inhibitory response to one color on the axis, but an excitatory response to the other. There are more complex patterns of response in color opponent neurons, but this is sufficient for our purposes.

Color opponent neurons have been found in many studies in honey bees. Honey bee studies were in fact foundational for our understanding of color opponent mechanisms in invertebrates. The classic study was by Kien and Menzel (1977b), and it is useful for illustrating the basic results. Figure 6.8 shows a neuron that is excited by green (long wavelengths) but inhibited by UV (short wavelengths). UV opponent interneurons (those excited by UV and inhibited by either green or blue) were also found. Later studies have continued to find color opponent interneurons in the

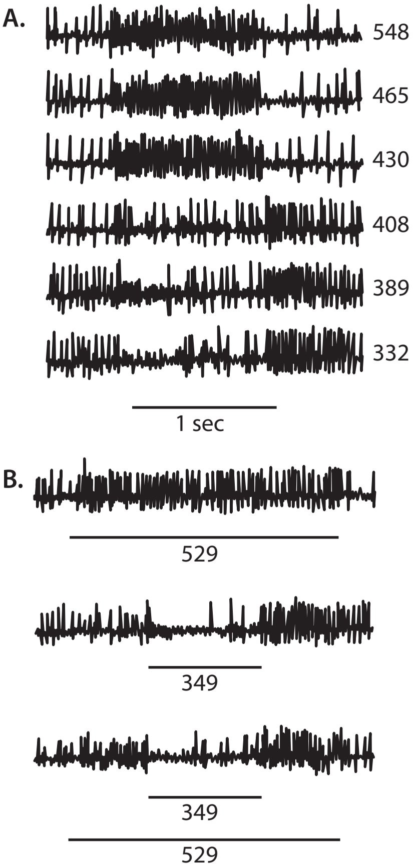


FIGURE 6.8. Color opponent neurons in the honey bee optic lobe. (A) Firing rate of interneurons in response to monochromatic light of various wavelengths. The neuron is excited by long wavelengths and inhibited by short wavelengths. (B) Response to excitatory and inhibitory light either alone or in combination (*from Kien and Menzel, 1977b*).

same regions of the optic lobe (Hertel, 1980, Hertel et al., 1987, Backhaus, 1991). Overall, there are now thought to be two color opponent axes: UV versus blue and green, and blue versus UV and green. In general, there is lot of elemental knowledge of neural mechanisms of color processing, but the physiological data are not yet sufficient to explain the sophisticated discriminatory abilities revealed by psychophysical behavioral studies. Further, there are many physiological results, including in bees, that do not fit well into the established paradigms (Briscoe and Chittka, 2001). Such considerations go beyond the scope of this work, however.

The second idea is that of a color space in which variation in primary colors form axes and all other colors are mixtures of these primaries (Helmholtz, 1896, Schrodinger, 1920). The neurobiology problem for this idea is to translate this conceptual model to a mechanistic one. Models take as raw input the number of photons received by each receptor and transform them with a mathematical function into an output that delineates colors. Several different mathematical functions have been proposed for bee vision (Backhaus, 1991, Chittka, 1992, Vorobyev and Osorio, 1998, Vorobyev et al., 2001). The models of Vorobyev currently fit the empirical data best.

The Honey Bee Clock

Molecular Genetics

Figure 6.9 shows the central clock for some well-studied insects. We focus first on the fly clock, as it is the best understood and is useful as a reference point. Generally speaking, animal molecular clocks are transcription/translation feedback loops of transcription factors that are paced by posttranslational modifications (PTMs) to generate a 24-hour cycle (Dunlap, 1999, Allada et al., 2001, Helfrich-Forster, 2005). In the fruit fly, expression of period (*per*) and timeless (*tim*) are controlled by clock (*clk*) and cycle (*cyc*). *Clk* and *cyc* dimerize and bind to the promoter of *per* and *tim* late in the day and early in the evening. The mRNAs from *per* and *tim* then enter the cytoplasm where they are translated into proteins. *Per* and *tim* protein levels peak in the middle of the night, and modulation of each by a series of phosphorylations (and other PTMs) pace the clock, eventually causing *per* and *tim* to dimerize and enter the nucleus to inhibit their own expression by interfering with binding of *clk-cyc* to the *per-tim* promoters (Chiu et al., 2008, 2011). Inhibition of *per-tim* expression eventually leads to low enough levels of these proteins to remove

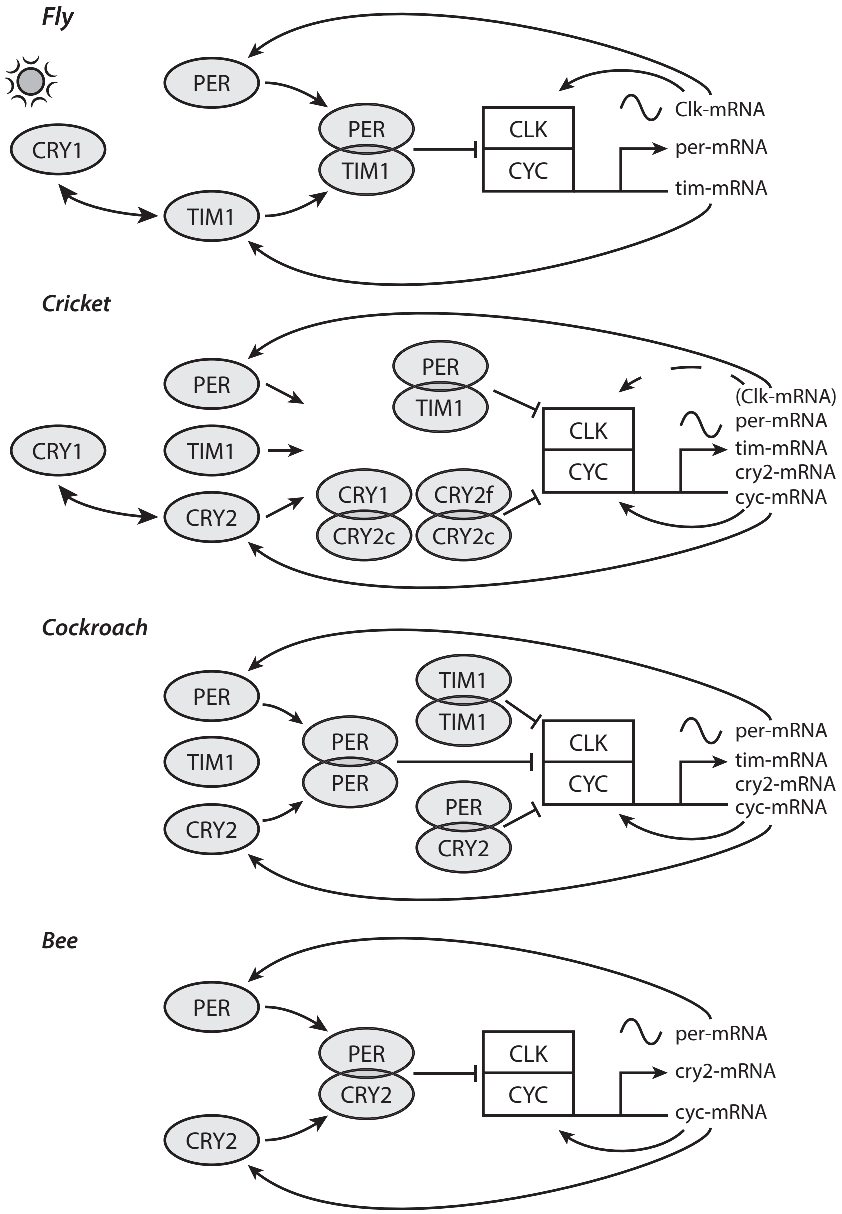

Fly
PER
CRY1
PER
TIM1
CLK
CYC
Clk-mRNA
per-mRNA
tim-mRNA
TIM1
Cricket
PER
PER
TIM1
(Clk-mRNA)
per-mRNA
CRY1
TIM1
CLK
CYC
tim-mRNA
cry2-mRNA
cyc-mRNA
CRY2
CRY1
CRY2c
CRY2f
CRY2c
Cockroach
PER
TIM1
TIM1
PER
PER
CLK
CYC
per-mRNA
tim-mRNA
cry2-mRNA
cyc-mRNA
TIM1
CRY2
PER
CRY2
Bee
PER
PER
CRY2
CLK
CYC
per-mRNA
cry2-mRNA
cyc-mRNA
CRY2

FIGURE 6.9. Insect molecular clocks (*redrawn from Beer and Helfrich-Forster, 2020*).

the inhibitory effect on *clk-cyc* and the feedback loop restarts. *Cry1* provides for entrainment by light, as it is photosensitive, and its activation leads to the degradation of *tim*.

If we contrast the fly clock with those of the other insects, we see strong similarity but significant differences (Beer and Helfrich-Forster, 2020). In crickets and roaches, for example, the clock has more components, but the same transcription/translation feedback loop is present. The honey bee clock is less well understood than for several other models, which is surprising given the bee's amenability of study and fascinating chronobiology (Moore et al., 1998, Bloch, 2010). What is clear is that *tim* is missing in bees as is *cry1*. *Cry2*, which is not light sensitive, takes the role of *tim*. *Cyc* also participates in a second feedback loop in bees, which is not well understood. How sunlight entrains the clock is also not known. Finally, with respect to the members of the central feedback loop, the bee clock is more like the mammalian clock than is the fly clock (Rubin et al., 2006).

Neurobiology of the Clock

The fly central clock is composed of four neuronal clusters: two clusters of ventral lateral neurons (ventral lateral neurons and dorsal lateral neurons) and two dorsal groups (Yao and Shafer, 2014). Although it is still poorly understood, the ventral lateral neurons seem central to the morning peak in fly activity, while the dorsal ventral lateral neurons are more associated with the evening peak (Yao et al., 2016). The dorsal neurons modulate activity in the lateral neurons, coordinate output of the clock to other brain regions, and are also important for the basic, free-running function of the clock in a poorly understood manner (Yao et al., 2016). There is feedback across the network via the neuropeptide, PDF, which is the central neuromodulator of clock neurons and the main neuromodulator used in the clock output to other brain centers (Beer and Helfrich-Forster, 2020). The output of the clock is vast and reaches into most neuropils in the brain. The bee clock has a similar structure (Fuchikawa et al., 2017, Beer et al., 2018). The main difference between the fly clock and the bee clock is that the bee clock is larger, with about 400 neurons versus 150 for the fly, and has more extensive projections to the higher brain centers, particularly the MBs. This is consistent with the more elaborate timekeeping sense of bees, but functional studies validating this idea are lacking. Thus far, PDF has been shown to cycle in concentration through the day in both nurses and foragers, and injection of

PDF into the bee brain caused a phase delay in circadian rhythm (Fuchikawa et al., 2017, Beer et al., 2018).

Neurogenomics

Sinha et al. (2020) has recently stressed the need for integration of genomic analysis of the brain (neurogenomics) with the more traditional neurobiology focused on neural networks. We saw the importance of transcription, for example, in our review of memory, and this fact is at the center of the field of sociogenomics, a termed coined by Gene Robinson, whose laboratory has pioneered the collection of such data in honey bees. In this final section of the chapter, we review some of the core findings of this field. The reader can find more work on this topic in chapter 12, on division of labor.

Broad Studies in Honeybee Neurogenomics

Quite a few studies have explored basic patterns in neurogenomics. These either sequence the transcriptome of key neuropils in a broad search for differentially expressed genes of interest, or they document, in broad terms, the importance of some classes of genes for proper brain function. Brockmann et al. (2009), for example, conducted a quantitative proteomics study to characterize the use of neuropeptides in the bee brain. Honey bees have over 100 of these neuropeptides (Hummon et al., 2006), which act as neurohormones, neuromodulators, or neurotransmitters. They identified about 50 neuropeptides in the brain and recorded differences in the expression of some for well-studied traits, such as caste differences or foraging choices. A study with a similar goal (to establish the general importance of a class of molecular actors) was conducted by Greenberg et al. (2012) on microRNAs. They identified many differentially expressed micoRNAs in the brain, some of which were candidates for a role in generating nursing behavior. With respect to the study of the transcriptome of key neuropils, several studies have explored MB function (Velarde et al., 2009, Lutz and Robinson, 2013, Shpigler et al., 2019). Velarde et al. (2009), for example, showed that the MBs are responsive to juvenile hormone and ecdysteroids, suggesting a common endocrine regulation of behavior across the body. Traniello et al. (2019) recently showed that different populations of KCs in the MBs respond to positive or aversive stimuli. This was based on expression of immediate early genes (IEGs) after exposure to an intruder (a negative encounter) or the queen (a positive social experience).

Studies of Individual-Level Variation

A series of studies has explored the basis of differences in aggression using isopentyl acetate (IPA), the main component of alarm pheromone, to trigger aggression. An initial study showed that IPA triggers the expression of the IEG *c-Jun* in the AL (Alaux and Robinson, 2007). *C-Jun* is a transcription factor involved in the early phases of olfactory learning and memory in mammals. A follow-up transcriptomic study found changes in gene expression in many genes in the brain after IPA exposure and between Africanized and European bees (Alaux et al., 2009c). This suggests that those genes that underlie aggressive responses are also those that have been changed by selection to modulate this trait across populations. A gene ontology analysis of the differentially expressed genes responding to IPA found a decrease in oxidative phosphorylation. Li-Byarlay et al. (2014) followed up by using pharmacological agents to decrease oxidative phosphorylation. This caused an increase in aggressiveness, providing a causal link. Chandrasekaran et al. (2015) later suggested, based on a metabolomics study, that aerobic glycolysis, which changes neurotransmitter levels, creates a more excitable neural state conducive to aggressive behavior.

Two studies have explored the neurogenomic basis of scouting in honey bees (Liang et al., 2012, 2014). Bees scout in two contexts: for food and for nests. A common transcriptomic basis for both was found in the neurogenomic state of the brain. Further, bees could be identified from transcriptomic data using expression patterns in just 89 key genes. Octopamine and glutamate were both found to increase the likelihood of scouting, while dopamine decreased scouting. Thus, the reward system plays some role in reinforcing the novelty seeking behavior exhibited by scouts.

Future Directions in Neurogenomics

Neurogenomics has a bright future thanks to the advent of two technologies: CRISPR/Cas9 and single-cell transcriptomics. CRISPR/Cas9 allows for the production of transgenic animals in nonmodel systems. The inability to create such animals has always hindered research on bees. The reader of this chapter may have noticed, for example, that a process such as olfactory learning is based on activity across several neuropils, each with a complex structure. Given the specialization of neuropils, and their varied use of signaling pathways, whole-brain extractions are limited in the light they can shed. Sequenc-

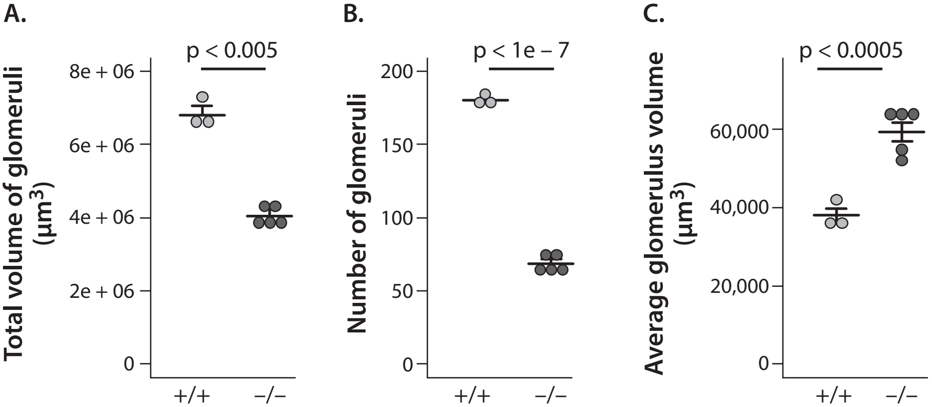


FIGURE 6.10. *Orco* knockdown mutants have smaller and fewer glomeruli (*from Chen et al., 2021*).

ing of single neurons across neuropils therefore allows for better integration of neurogenomics work with neurobiology focused on neural networks.

A strong start has been made on using both approaches in bees. For example, using CRISPR/Cas9 to knock out the nonspecific olfactory receptor *orco* showed developmental changes (fewer glomeruli in the AL and changes to glomerular size) along with extensive transcriptomic changes (in OR levels) in the antenna, as shown in figure 6.10 (Chen et al., 2021). With respect to transcriptomics, Traniello et al. (2020) explored the cause of precocious foraging in bees infected with deformed wing virus. A previously generated brain transcription factor gene regulatory network predicting foraging behavior also showed a strong association to the neurogenomic state of young, infected bees (Shpigler et al., 2019). This suggests that these bees have physiology more consistent with foragers than nurses. Many studies have now shown this connection between infection, by many pathogens and parasites, and altered adult behavioral development (Amdam et al., 2004a, Goblirsch et al., 2013, Perry et al., 2015, Natsopoulou et al., 2016). They then used single-cell transcriptomics to show that it is expression in glial cells that drives this similarity (Traniello et al., 2020).

7

Neuroethology and Cognitive Science

There are gray areas between the major branches of neuroscience (neurobiology, neuroethology, and cognitive science) that make it difficult to classify a particular study as belonging more to one category than another. The choice of which topics to cover here, instead of in the last chapter, is thus somewhat arbitrary, but they all focus on neuroscience results achieved in biologically realistic contexts, or on the practical consequences of the bee's sensory and cognitive systems.

The Visual System

The honey bee has played a prominent role in the study of invertebrate vision. This is a subject with a rich history, which we unfortunately do not have space to cover. The interested reader is encouraged to look for work prior to von Frisch in Lubbock (1881), Turner (1910), and Giurfa and Sanchez (2020). Color vision in bees was demonstrated in a series of studies in the early 1900s by von Frisch (reviewed in von Frisch, 1967), who showed that bees respond to color and not brightness. His classic experiment involved training bees to a feeder set over a colored card and then replacing the single feeder with an array of feeders over cards, some of which matched the original card's brightness but not color. Bees always returned to the original color no matter its spatial position in the array. Great progress was made after von Frisch's pioneering work. Kuhn (1924) showed that bees can see UV light, and Daumer (1956) demonstrated that bees are trichromats. Daumer's conclusion followed from the demonstration that bees have three peaks of sensitivity in their color vision, corresponding to their three photosensitive pigments (Autrum and Vonzwehl, 1964).

What Bees See in Practical Terms

RESOLUTION, OR VISUAL ACUITY

Two aspects of the compound eye strongly affect resolution. These are the light acceptance angle of an individual ommatidium and the angular divergence of the optical axes of neighboring ommatidia, or the interommatidial angle (figure 7.1). The acceptance angle (the arc over which light is received) sets the size of the pixel produced by each ommatidium (to use an analogy to your computer monitor). The interommatidial angle defines the direction each ommatidium is facing and hence how the pixels are arranged across space. These two angles, acceptance and interommatidial, can be modulated evolutionarily such that the whole visual space is well sampled. Theoretical work considering these and other factors has calculated a maximum resolution for the honey bee of about 1.4°, which agrees with empirical behavioral tests (Laughlin and Horridge, 1971, Snyder, 1979, Srinivasan and Lehrer, 1988). To put this resolving power into perspective, humans have well over a hundred times better resolution than bees (Land, 1997). In more personal terms, the disk of the full moon is about 0.5° wide, as is that of the sun. Thus, the best a bee can do is to sample the average light intensity in an area of space about three moons wide by three moons tall. Hence, when they are using their sun compass, only one ommatidium is looking at the sun.

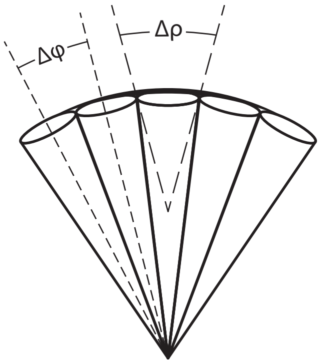


FIGURE 7.1. Interommatidial angle (ϕ) and acceptance angle (ρ) of the insect compound eye (*redrawn from Vorobyev et al., 1997*).

With respect to resolution and motion, this is often measured by electrical recordings from the retina as a flickering light is shown. The highest frequency that the retina is able to match is called the critical fusion frequency (CFF). For honey bees, the CFF is 165–300 Hz, while it is 20–70 Hz in mammals, making the bee visual system about five to six times faster than our own (Autrum and

Stoecker, 1950). Hence, bees likely see motion with far better clarity (less blur) than we do (Srinivasan and Bernard, 1975, Srinivasan and Lehrer, 1984).

OTHER BEHAVIORALLY RELEVANT ASPECTS OF BEE VISION

Bees have a nearly 360° field of vision (with only a small blind spot behind them) that includes three regions of binocular vison (Seidl and Kaiser, 1981). Binocular vision only extends a few centimeters, however, suggesting it may not be behaviorally relevant (Srinivasan, 2011a). An important consequence of the bee's compound eye design relates to vision in low light. Because the ommatidia are surrounded with screening pigment that blocks light rays coming in from unwanted angles (and thus limits the area over which light is collected), the ommatidium does not function well in dim light and quite poorly at night (Land, 2018). With respect to the practical consequences of color vision, we already mentioned that bees see into the UV and that they can determine the plane of polarized light. We discuss the role that polarized light plays later in the context of navigation. UV is mainly of importance as some flowers have nectar guides (dark area in their centers with UV-absorbing pigment), surrounded by UV reflecting patterns (figure 7.2; Thompson et al., 1972).

We conclude with a discussion of whether bees can see the frequencies that we perceive as red. The absorbance spectra of their opsins suggest they cannot differentiate it from other colors, but that is not the same as whether they can see it or not. This distinction has two components, made clear in a paper by Chittka and Waser (1997). First, red colors in nature are not monospectral. Flowers that appear red to us reflect long wavelength light, but they also often reflect the blue and UV wavelengths. Bees can thus respond to the wavelengths they can see, even if this is a small part of the total reflectance. Hence, strikingly red flowers (to us), such as bee balm *Monarda didyma*, may actually appear blue to bees. Second, the bee's green opsin, with a peak sensitivity at 544 nm, has 50% sensitivity at 594 nm, which appears orange to us, and it still retains about 10% sensitivity at 630 nm, which is orangish red. There is, however, no overlap with the input from another receptor for these wavelengths, meaning they cannot discriminate colors, only brightness. Bees can therefore sense the frequencies we perceive as red, although they cannot differentiate different wavelengths in this region of the spectrum. Finally, even for flowers that appear to us as red that are more or less monospectral, and for which the bees cannot see directly, they can still see them as black against a green back-

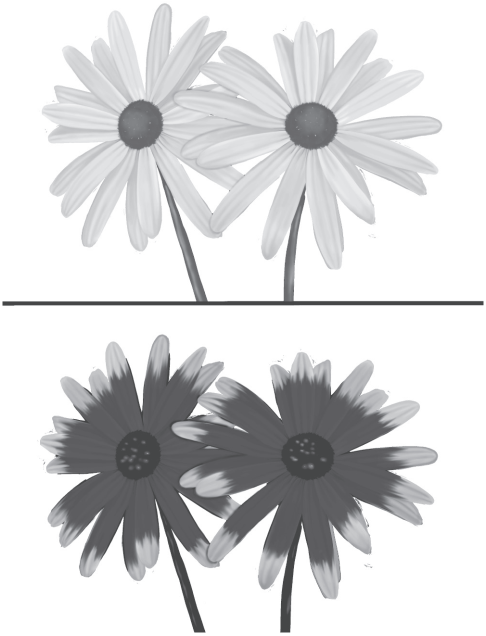


FIGURE 7.2. An example of a nectar guide. (A) The flower in visible light (to humans). (B) The flower in UV light (*redrawn based on Thompson et al., 1972*).

ground. Thus, no matter the context, bees can see the flowers we see as red, although, of course, they do not perceive them in the same manner we do.

Visual Learning

We focus on the learning of achromatic patterns in this section (rather than the learning of color patterns), as this field is more involved conceptually, maybe because such patterns are easy to manipulate experimentally. With respect to color, we simply note that bees have a bias for colored objects in the blue range in the shape of flowers (Menzel, 1967, Chittka and Menzel, 1992, Giurfa et al., 1995, Lehrer et al., 1995, Lunau and Maier, 1995, Howard et al., 2019b, Koethe et al., 2020). Bees also learn colors much faster than achromatic patterns, at least when using absolute conditioning (Giurfa, 2004). Bees also have color constancy, meaning they do not take brightness into consideration when identifying a color (Werner et al., 1988, Lotto and Wicklein, 2005). Finally, bee decisions are characterized by a trade-off between speed and accuracy, particularly in the context of color pattern discrimination (Chittka et al., 2003). In short, bees take longer to make cognitively difficult decisions such that this imparts a cost. The benefit of the decision thus must outweigh this cost in order for it to be adaptive for the bee to attempt the discrimination task.

The study of pattern learning, as for many topics, goes back to pioneering studies by Karl von Frisch (von Frisch, 1915, Hertz, 1929). His approach was to train bees to associate food with a black-and-white pattern beneath the feeder and then to present the pattern among many others randomized in space. The bees could choose the correct pattern no matter where it was placed on the table and in spite of the fact that all the patterns were presented with food. A limitation of von Frisch's approach was that by laying the pattern down on the table, the angle at which the bee approached it could not be controlled. Thus, the same pattern could look different when approached from different directions and heights.

Researchers following von Frisch's preliminary work used patterns that the bees could see on a vertical screen head-on as they approached (Wehner, 1967, 1972, Lindauer, 1969). During this period of research, it was shown that bees can learn to recognize (be trained to visit) many patterns common in flowers, such as those exhibiting radial or bilateral symmetry (Wehner, 1967, 1971, 1972, Gould, 1985, 1986b). It was also noticed that bees tend to hover for a second or so in front of the image before landing (Wehner, 1977). This gave rise to the idea that bees memorize (take a snapshot of) the whole image. We cover the now extensive evidence for this in the section on navigation. In general, this phase of research was focused on the rigid learning of patterns, a sort of pattern recognition form of rote memorization.

Later research in visual learning sought to determine if bees could form generalizations. In other words, they can clearly recognize a particular radial pattern, but could they be trained to learn to visit any radial pattern? This suggests to some that a bee is capable of generalizing her knowledge. A pioneering study by Wehner (1971) trained bees to associate a black or white bar at an angle (against the opposite background) with a food reward. After bees learned to do so, they were then given the choice of bars with similar angles (but with different bar lengths or colors) versus various control patterns. Bees chose the pattern with the same angular orientation as the one they were initially trained to associate with a reward. Many similar studies followed with a diversity of patterns (Vanhateren et al., 1990, Horridge and Zhang, 1995, Giurfa et al., 1996a, 1999, Horridge, 1997, Neal et al., 1998, , Efler and Ronacher, 2000, Chen et al., 2003).

Bees can learn to generalize with respect to symmetry, either radial or bilateral, for example, and other spatial patterns such as disk versus ring. They can also generalize based on color and size (Ronacher, 1992, 1998).

The current concern in this field is to test the bee's ability to solve more complicated and abstract problems. For example, bees are poor at learning a camouflaged pattern, such as a shape (a circle, for example) embedded in a similar color. However, when Zhang and Srinivasan (1994) first trained bees to recognize the shape against a white background, and then trained the bees to recognize the pattern against a camouflaged background, they were better able to do so. This suggests that past experience modulates the ability of bees to learn (Zhang et al., 1995). Studies have also looked at how well bees can be trained to fly though mazes (Zhang et al., 1996, 2000). Bees can learn the path through a maze that is marked with visual landmarks along the way after about 20 trials. Studies of the sophistication of the bee's short-term memory have also been conducted. Bees can be taught to remember a previously seen image and match it to the current stimulus (Zhang et al., 2005). They can remember colors, patterns, and odors and can also generalize in this context. Bees can also learn to form pairwise associations when presented with multiple stimuli (Zhang et al., 1999, Giurfa et al., 2001). When they are presented with two images (A and B) and then another two afterward (C and D), for example, and are rewarded only if they can associate A with D, they can readily do so. Bees can also combine cues across sensory modalities, learning to associate odors with visual cues to navigate the maze. Finally, bees may have a sense of quantity (Chittka and Geiger, 1995, Howard et al., 2018, 2019a). Dacke and Srinivasan (2008a) showed, for example, in flight tunnels, that bees can remember up to four visual stimuli. Studies in which bees were tested for their ability to match patterns (in this case, the number of objects in the rewarded stimulus) also indicate an ability to match up to four objects (Gross et al., 2009). However, it is still unclear if these studies explore an ability to comprehend discrete units (numbers) or continuous variation (magnitude) (MaBouDi et al., 2021).

Olfactory System

Olfactory Learning

The earliest studies of olfactory learning again go back to von Frisch's work (reviewed in von Frisch, 1967). His by now familiar paradigm for testing preferences—training bees to a feeder associated with the stimulus and then testing their preference when returning to find an array of choices—was extended to odors. He found that bees can associate odors with rewards, are able to distinguish many compounds, and have memories that are robust and long lasting (von Frisch, 1919). He also showed that they can generalize their preferences, as they prefer similar odors when presented with different choices after training. Modern work uses the proboscis extension response (PER; also called reflex) paradigm, described below, to catalog the bee's impressive olfactory abilities and to probe their neuronal and molecular bases (Giurfa and Sandoz, 2012).

Early Work

The current approach to the study of honey bee olfactory learning traces back to two studies by Japanese researchers in the 1950s and 1960s (Kuwabara, 1957, Takeda, 1961). That hungry bees will reflexively extend their proboscis when their antennae or mouthparts are touched with sugar water was known since the 1940s (Frings and Frings, 1949). Kuwabara coupled this reflex with a Pavlovian conditioning protocol. In Pavlovian conditioning, a stimulus that does not normally elicit a particular response, the conditioned stimulus (CS), is paired with one that does, typically a reward, called the unconditioned stimulus (US). Over repeated exposures to both together, the animal learns to associate the CS with the reward such that it elicits the response. This simple form of associative learning, PER, is the basis for most of the work on honey bee olfaction and the neural basis of learning.

Kuwabara (1957) initially used color as the CS. His student, Takeda (1961), however, adapted the protocol for use with odors. In his studies, the bee's wings were clipped, but they were freely moving. The approach was to present the bee with an odor, then touch its antenna with a drop of sugar water, which it was allowed to consume. After a single trial, many of the bees would extend the proboscis after the odor was presented alone, and after repeated trials nearly all did. Takeda's study then focused on testing some of the basic Pavlovian patterns first found in mammals (Pavlov, 1927). These included extinction (learning that a learned CS is no longer rewarded), conditioned inhibition (suppressing a conditioned response depending on context), and second-order conditioning (learning to associate a second odor with the first CS), all of which are exhibited by bees. A weakness of this early work was a lack of proper controls and low sample sizes (these problems were common to the day). These issues were remedied by Bitterman et al. (1983),

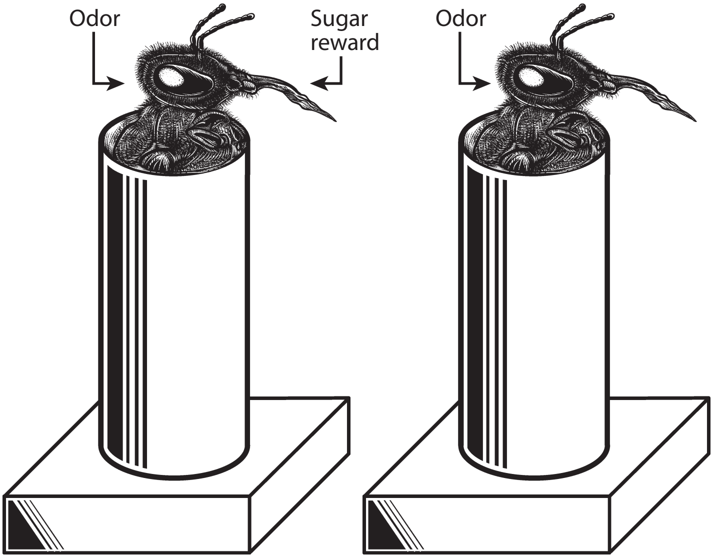


FIGURE 7.3. Bee undergoing PER (*redrawn from Matsumoto et al., 2012*).

who standardized the protocol and verified that the bees were doing Pavlovian associative learning and not operant conditioning. Subsequent work is largely based on this last study. Figure 7.3 shows a bee undergoing PER within this experimental paradigm.

Olfactory Resolution Studies

DISCRIMINATION AND GENERALIZATION

Following the pioneering early work, most PER studies focused on determining the bee's ability to discriminate between odors and to generalize (recognize similar odors). Vareschi (1971), for example, explored the bee's ability to recognize and generalize across many odors. He found an impressive ability to identify odors, alone or in combination, and found that generalization is based on compounds either having the same functional groups (alcohols, ketones, aldehydes, etc.) or similar chain length. Later work confirmed these results (Laska et al., 1999, Smith and Menzel, 1989) and culminated more or less in a study by Guerrieri et al. (2005). Here the bee's responses to 16 compounds, which varied in side group and chain length, were explored in a fully combinatorial fashion. That is, the generalizability (how likely bees are to respond to odor B after learning A) was explored for all pairwise comparisons. This work confirmed that bees use side groups and chain length as major determinants in olfactory discrimination. However, there was much idiosyncratic complexity. Aldehydes and alcohols, for example, showed no chain length effects, although ketones did. The nature of generalization within a functional group class was also not consistent across different functional groups. A principal component analysis found that chain length was the most explanatory variable for the generalization response, with functional groups being the second and third principal components. It is unclear if this result depends on the particular compounds used.

MIXTURES AND KEY COMPOUNDS

A large body of work has explored odor discrimination based on the behavioral ecology of the interaction between bees and flowers. Hence, these studies stress the use of floral odors. Two early studies, for example, explored the compounds important for the olfactory identification of oil seed rape odor (Phamdelegue et al., 1993, Blight et al., 1997). It was found that of the many compounds in the blend (up to 16 tested) some contributed more to the response than others. This gave rise to the notion of key components, which are thought to dominate blends (Laloi et al., 2000). Some work has explored what makes some compounds key and others not, but it is still poorly resolved. Reinhard et al. (2010) showed that volatility or chemical structure does not determine being a key component, although concentration in the blend does. More recently, Schubert et al. (2015) found that odors associated with less generalization (thus more distinct) tended to be key odors. It would be good to replicate this conclusion with more complex mixtures that allow for more intense inhibition via the LNs. Chan et al. (2018), for example, recently found that complex odors may be more efficiently processed than the pure compounds often used in this field.

Orientation and Navigation

Orientation Flights

Probably the most obvious behavior associated with navigation are the orientation flights that young bees make prior to beginning their foraging careers (Lehrer, 1991, Capaldi and Dyer, 1999, Capaldi et al., 2000, Degen et al., 2015). These consist of a series of short flights, usually taken at the same time each afternoon. The bee leaving the nest for the first time begins by turning around in flight a few meters from the entrance to face the nest. She then flies back and forth in ever increasing arcs, which eventually move away from the immediate nest area. As expected, experimental work has shown that bees are learning the landscape (landmarks) near the nest during these flights. A recent study, for example, captured bees after their first orientation flight and released them in a place either within the area they had scanned or outside of it but equidistant to the nest. Those bees released within the area already surveyed flew immediately home while those released outside of it got lost and most did not return home (Degen et al., 2016). Orientation flight behavior also occurs among experienced foragers when a colony is moved to a new location either naturally by swarming or artificially by a beekeeper (Degen et al., 2018).

Path Integration

Generally speaking, an animal can navigate either with or without knowledge about the outside world. To navigate without knowledge of the landscape, the animal can use path integration, also called dead reckoning (Anholt, 1994, Wehner, 2003, Wehner and Srinivasan, 2003). In simple terms, if one moves in a straight line, then turns a known angle and moves further along another line, we have the classic side-angle-side problem we learned as trigonometry students. The line connecting the endpoint with the beginning can then be computed as it closes the triangle. Thus, if an animal is continually monitoring its distance traveled along with its turns, then it can also set a vector (distance plus direction) home. The clearest data demonstrating path integration are from desert ants in the genus *Cataglyphis* (Wehner, 2020). In the basic proof, an ant is picked up at a food source after feeding and dropped down a set distance to the south. The ant is then observed to set off on the vector that would have taken her home if she had not been moved (Muller and Wehner, 1988). The ant also travels what would have been the appropriate distance before realizing she is lost, at which point she begins a local search. Bees do path integration as well, although they are less reliant on it than are these particular ants. We return to this in the section on cognitive maps (Gould, 1986a, Menzel et al., 2005).

Given the nature of path integration, the obvious questions are, how do bees know how far they have gone and how do they know what direction they are going in? Both of these problems have been the subject of much study. We discuss the problem both in terms of path integration and the waggle dance (since bees use path integration to inform the dance).

HOW DO BEES DETERMINE DIRECTION? THE SUN COMPASS

The sun rises in the east and sets in the west. There is thus azimuthal directional information in the position of the sun at those times. If one can compensate for the movement of the sun, then its position can be used as a directional reference at any time. This is known as a time-calibrated sun compass. Conclusive proof for use of such a sun compass was obtained with clock-shifting experiments (Lindauer, 1954, 1960, Renner, 1959). Here the bee's clock is phase shifted by a known amount, allowing one to predict the direction of (incorrect) travel that would result from using a sun compass. Much detailed work followed these early studies exploring exactly how the sun is used and tracked (Dyer and Gould, 1983). The core result was that bees use the sun's azimuth as the reference. The interested reader is encouraged to consult Dyer and Gould (1983) for a review of this work.

POLARIZED LIGHT

We do not see the plane of polarized light as an independent property from wavelength and intensity, but many other organisms, including insects, can. Von Frisch discovered this early on, as bees can navigate using their sun compass when the sun is behind clouds. They can even do so when the sky is almost completely overcast. He was able to show that a patch of blue sky about 15° wide is sufficient. Further, when he put a polarizing filter in front of this patch, the dance angles changed (von Frisch, 1949, 1967). Studies have explored whether the sun or the polarized light is the main source of information to the bees (Brines and Gould, 1979, 1982). It turns out they use both sources concurrently because in nature they are not in conflict. When they are put into conflict by experimental manipulation, the bees prefer the sun but will use the polarization patterns if the signal from the sun is weak (Dyer and Gould, 1983).

HOW DO BEES CALCULATE DISTANCE?

Optic flow refers to the movement of images across the retina as an animal moves. An elegant set of experiments has shown that the bees calculate distance based on optic flow (Esch and Burns, 1995, 1996, Srinivasan et al.,

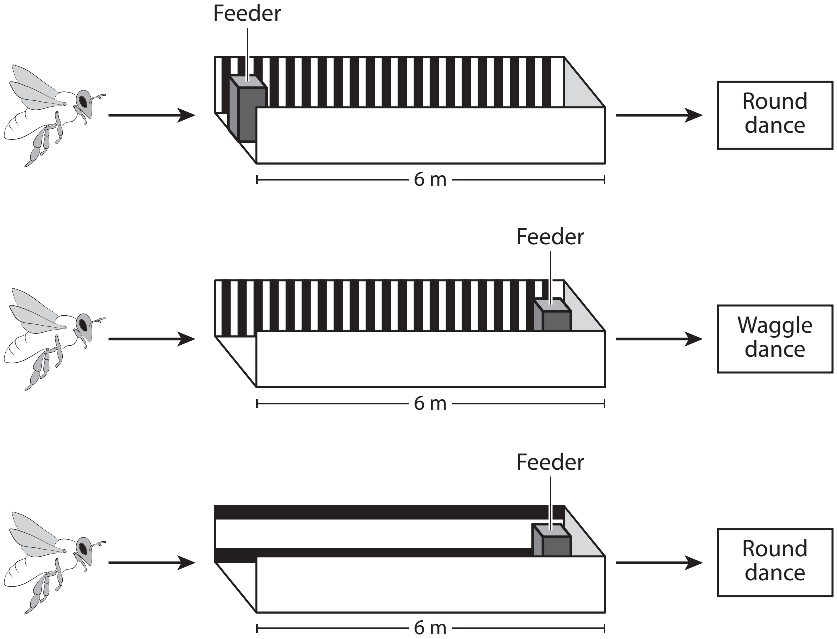


FIGURE 7.4. Bees use optic flow to measure distance. Top: A short flight leads to the round dance upon return to the nest. Middle: Flight down a six-meter tunnel with vertical stripes lead to an advertised distance of 200 m using the waggle dance. Bottom: The same distance traveled down the tunnel with horizontal stripes elicited a round dance, indicating a close distance to the nest. (*from Srinivasan, 2011a, adapted from Srinivasan et al., 2000*).

2000). The basic experimental design is shown in figure 7.4. The bee is trained to fly down a narrow tube to a feeder. Optic flow is varied by changing the pattern of black-and-white regions (stripes or splotches) inside the tube. In these studies, bees have been shown to vary the distance of their waggle runs (the basics of the waggle dance are covered in chapter 13) in accordance with variation in optic flow (Esch et al., 2001). Many follow-up experiments were conducted to rule out all the potentially confounding variables, such as flight time, energy spent, and other visual cues (Srinivasan, 2011a, 2011b). For example, when the stripes in the tunnel are parallel to the direction of flight, bees cannot judge distance (Si et al., 2003).

There is one last wrinkle in the story of path integration that is not well understood. When bees are made to fly a detour around an obstacle, or over a hill, to get to a food source, the direction of the waggle dance they produce corresponds to the bee line back to the hive (the path integration result for direction). However, the indicated distance (the time of the waggle run) includes the flight around the obstacle, or over the hill (von Frisch, 1967). There may thus be two separate path integrators, one for direction and another for distance, or two integrators that both track distance and direction, with one or the other being used depending on the context (Dacke and Srinivasan, 2008b).

Non-Egocentric Navigation

There are two approaches to navigation that are not mutually exclusive based on knowledge of the outside world. These are image matching and a cognitive map (Menzel et al., 1996, Collett and Collett, 2000, Collett et al., 2013). Image matching is well explored and noncontroversial. Cognitive maps are the source of much debate regarding insects, with honey bees at the center of discussion.

IMAGE MATCHING

Image matching involves an animal taking snapshots of the world and storing them in memory for future use (Cartwright and Collett, 1982, Collett, 1996, Collett et al., 2002), the use being as a template for comparison with the current image on the retina. Image matching falls into two general categories: alignment and position matching (Collett et al., 2013). They have to do with landmarks that are either far or near, although, of course, there is gray area (Kheradmand and Nieh, 2019).

We start with alignment matching. This refers to the bee learning the skyline (the pattern of mountains relative to the sky, for example) or the panorama (patterns of various sorts across the sky). If a bee takes multiple snapshots of the skyline, plus panorama, from all relevant angles as she approaches a goal (such as the hive), then she can use these stored images to guide the direction of her return flight by matching them later. This is thought to be important for navigation over long distances. For a long time, there was no clear support for this in bees, although there was much support in ants (Hölldobler, 1980, Harris et al., 2007, Graham and Cheng, 2009). However, a recent study showed that bees do in fact use the skyline in this way (Towne et al., 2017). By training bees to a feeder surrounded by an experimental skyline, the authors were able to show that, when the controlled skyline was rotated, the bees' departing directions changed accordingly.

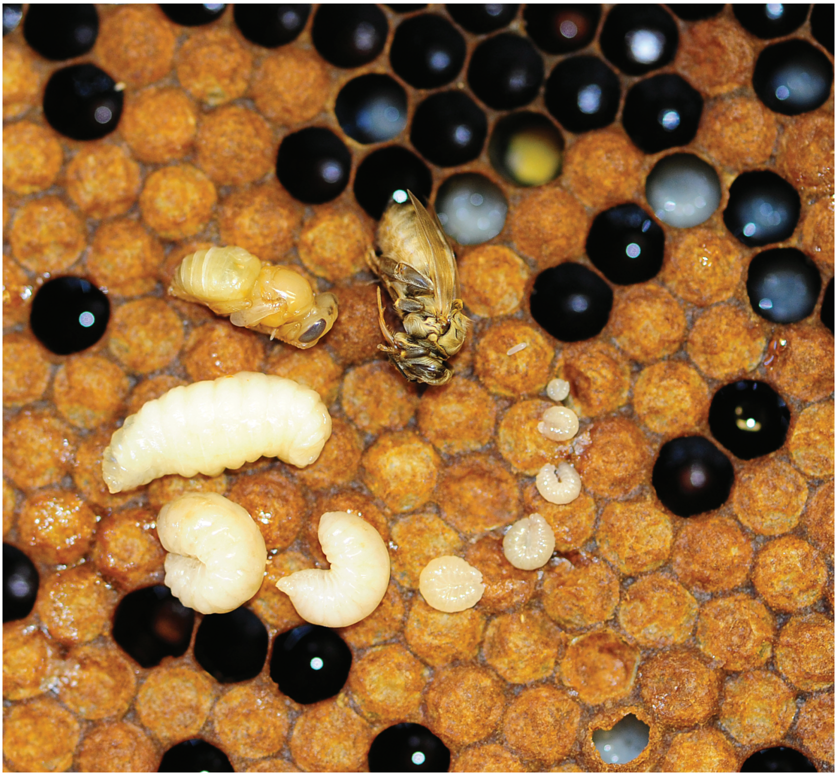


PLATE 1. Life cycle stages of the worker honey bee from egg to adult.

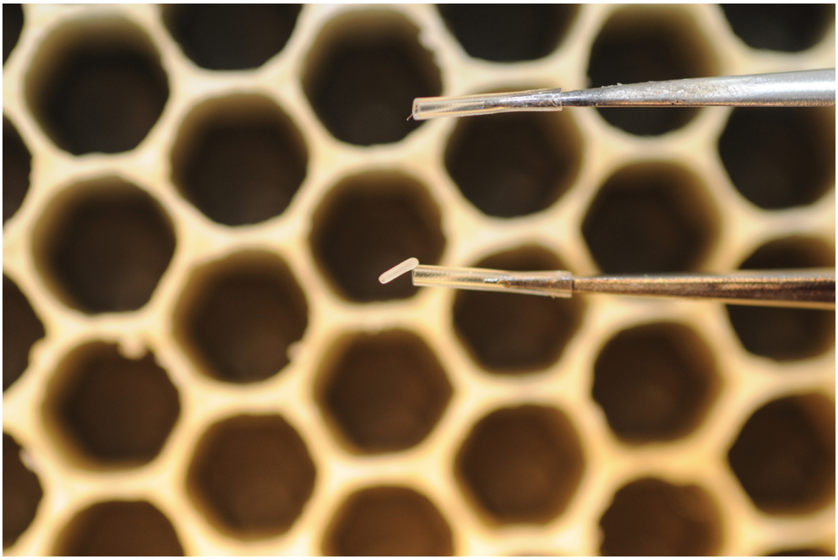


PLATE 2. Honey bee egg.

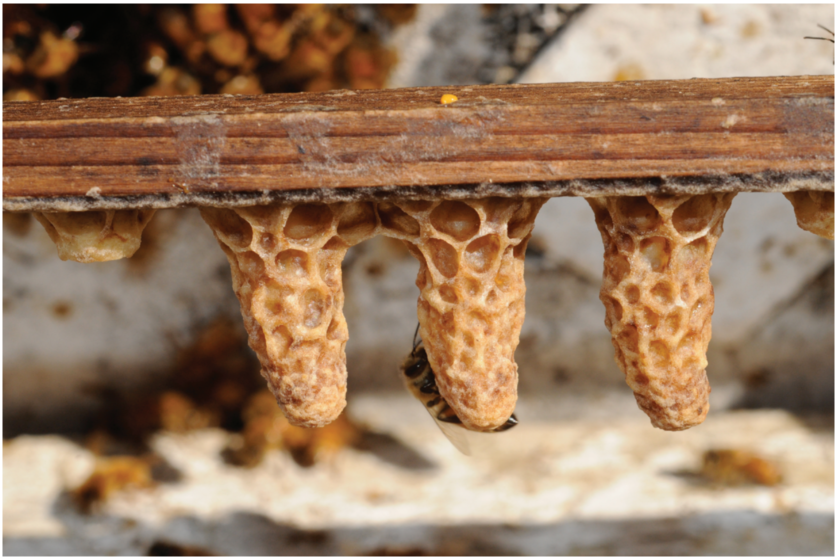


PLATE 3. Queen cups.

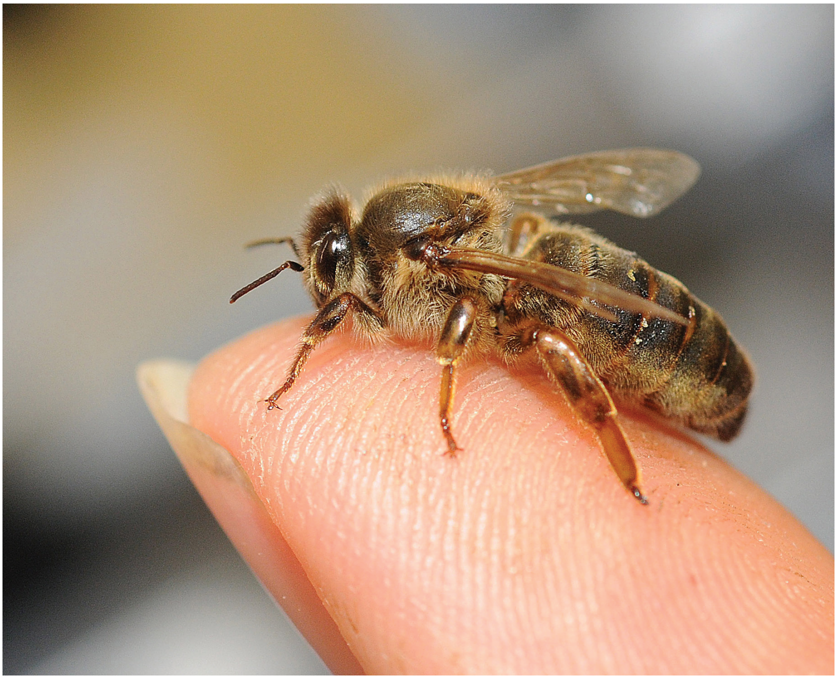


PLATE 4. Queen bee.

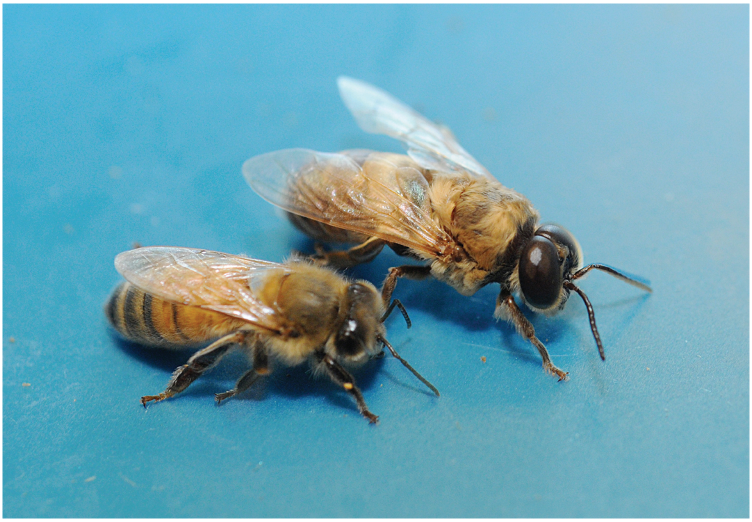


PLATE 5. Worker with drone.

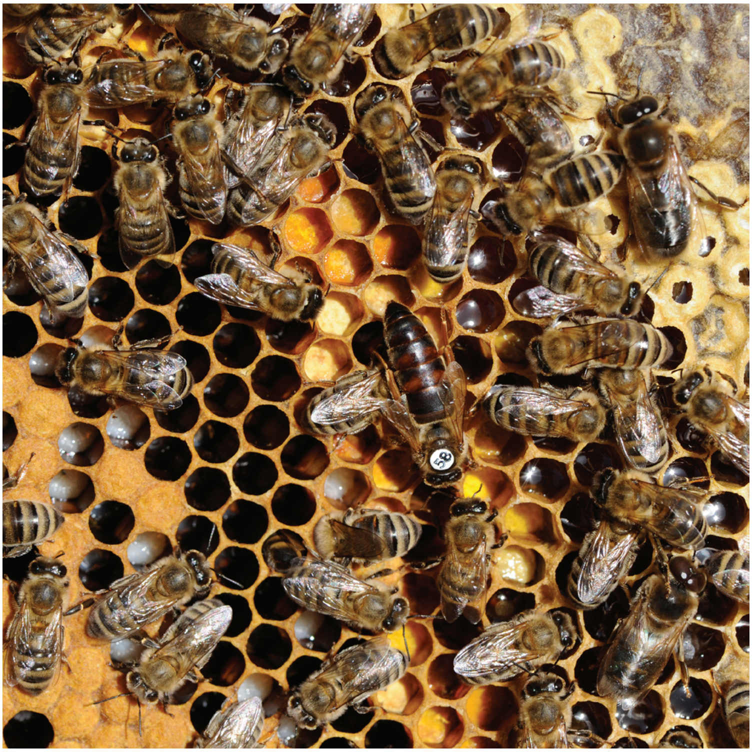


PLATE 6. All three honey bee castes inside the nest.

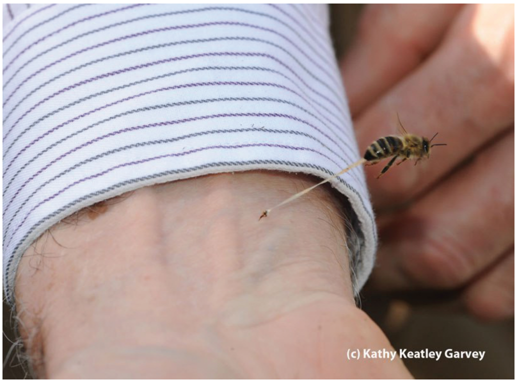

(c) Kathy Keatley Garvey

PLATE 7. Stinging honey bee.

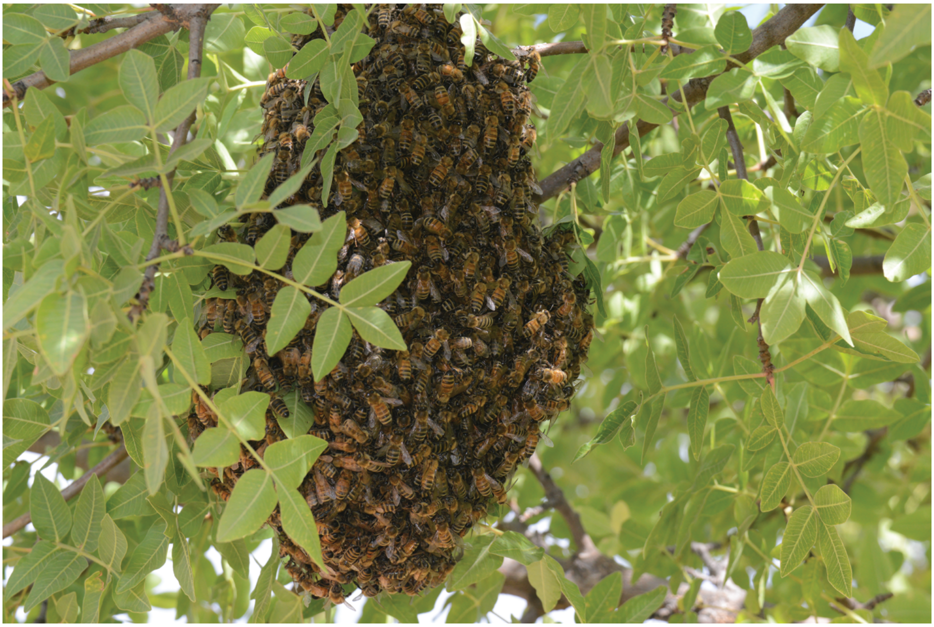


PLATE 8. A small swarm in a tree.

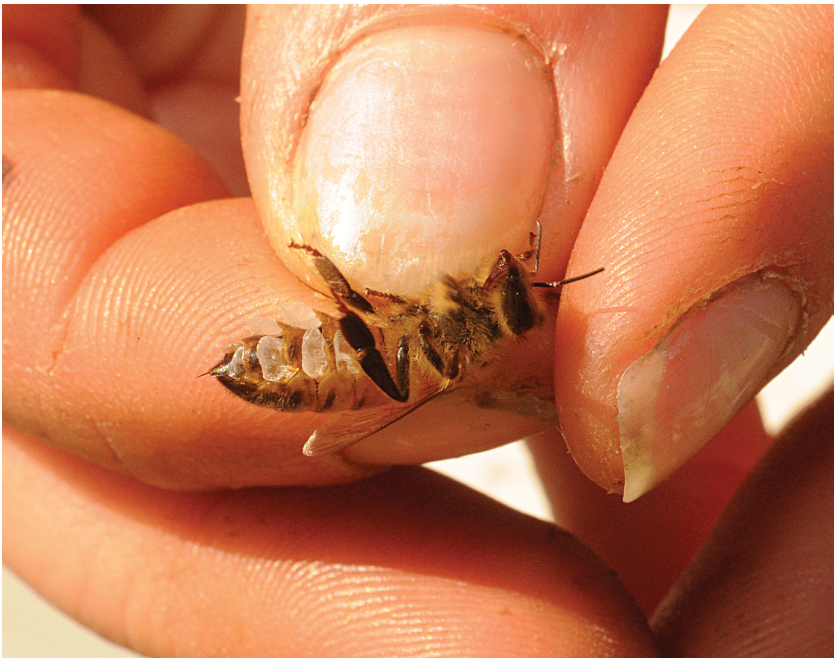


PLATE 9. Bee secreting wax flakes.

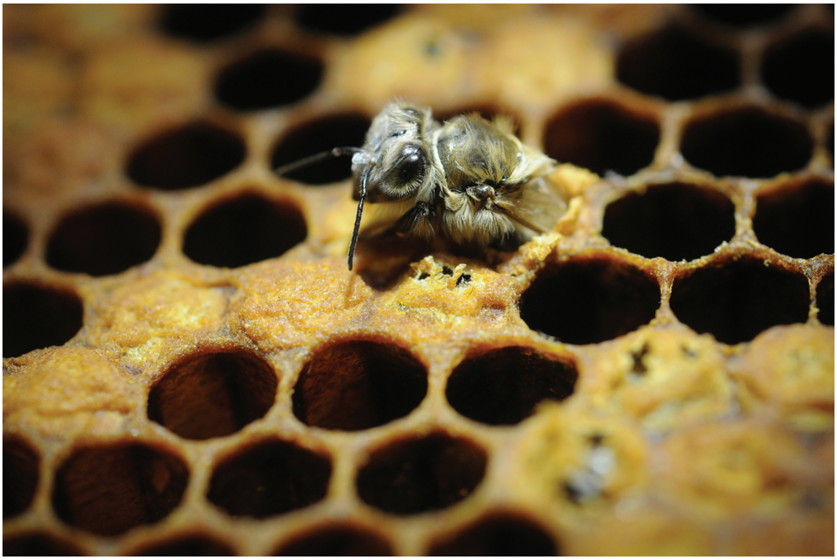


PLATE 10. Newly emerging bee.

PLATE 11. Bee foraging on lavender.

PLATE 12. Bees collecting water.

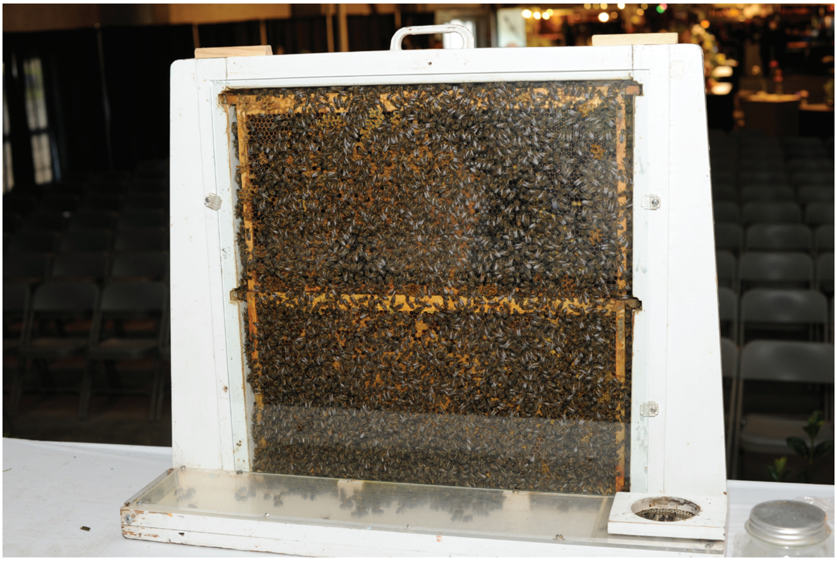


PLATE 13. An observation hive.

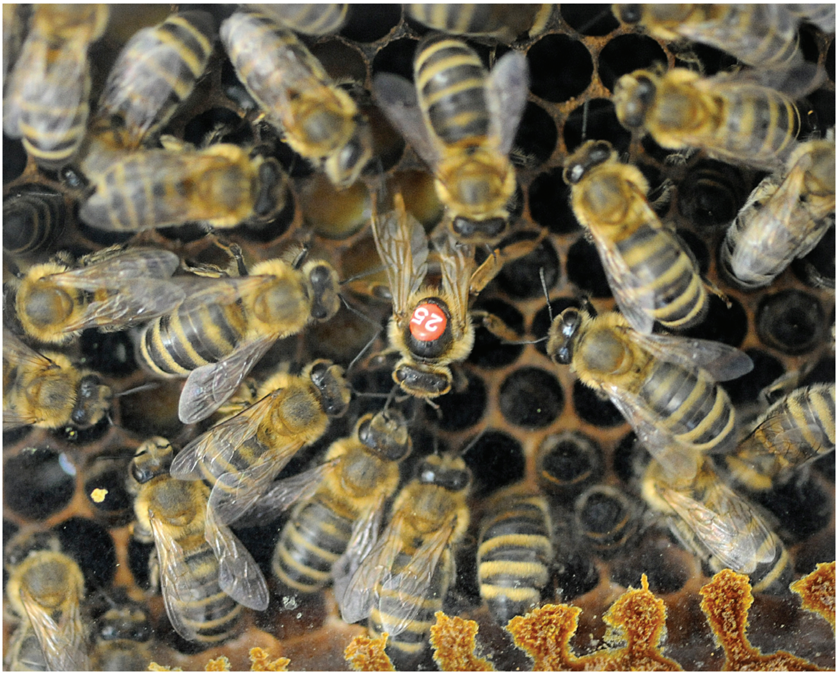


PLATE 14. Laying queen with retinue.

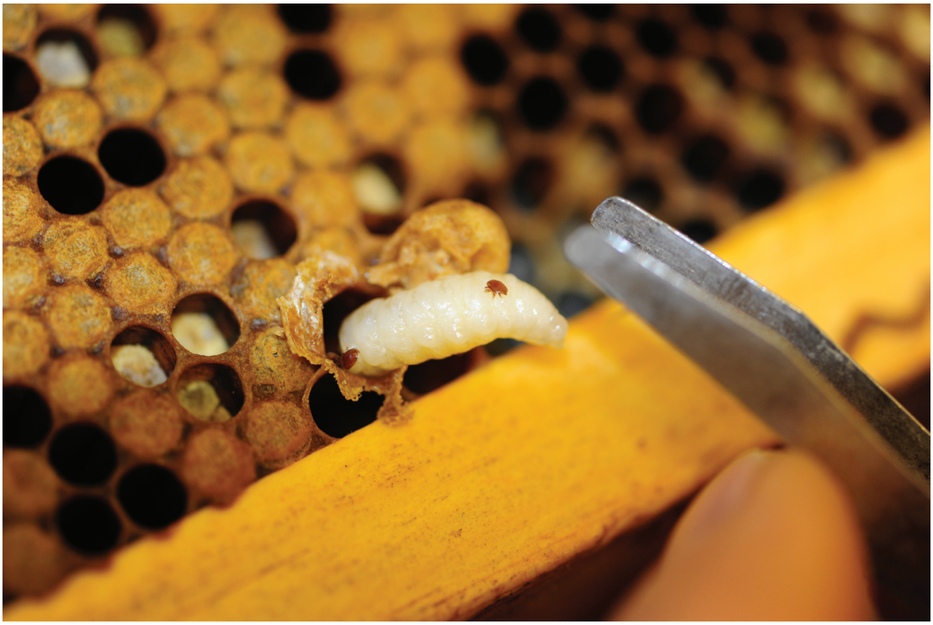


PLATE 15. Varroa mites on developing bee.

PLATE 16. Colonies being used for almond pollination.

There is considerably more work on position matching in bees (Collett, 2009, Collett et al., 2013). Here again the idea is that a bee stores an image on the retina and attempts to match the image to the current view. However, for near objects the size and orientation of the image on the retina are going to change rapidly as the bee varies her angle to the target and the distance. Matching could thus be used not only to fly at a given angle but also to judge distance and direction. This idea has a long history. Lindauer (1960), for example, showed that bees learn the precise location of a feeder in relation to local landmarks. Wehner (1972) conducted studies suggesting that when bees learn patterns they use the same ommatidia to view the same parts of the image. This suggests that bees store the image across the retina and attempt to match it (with respect to what is seen by each ommatidium), by moving relative to the landmark.

These early studies were greatly expanded on by Collett and coauthors. Collett's approach grew largely from the pioneering work of Tinbergen on digger wasps (Tinbergen, 1932). In a classic study, Tinbergen put three pine cones in a triangle around a digger wasp nest. After the wasp learned these cues, he then moved the three cones (in the same pattern) some distance from the nest. When the wasp returned, she flew to the center of the triangle (not her nest). She had thus learned her nest's location via its position relative to these local landmarks. Collett's early studies use a similar approach with more control and greater experimentation (Cartwright and Collett, 1982, 1983, Collett and Cartwright, 1983, Cheng et al., 1987). In one study, for example, bees were trained to fly to a whitewashed room with no local landmarks other than those supplied by the experimenter (Cartwright and Collett, 1982). In this situation, when the food source was placed near a single small local landmark, the bees quickly learned to search relative to the position of this landmark. When the landmark was made bigger or smaller, the bees searched further away or closer. This is suggestive of their using image matching to judge distance since a bigger object requires the bee to be further away to be the same size on the retina. Experiments with more complex arrangements of landmarks supported the same conclusions (Collett, 2009, Collett et al., 2013).

The study of image matching has involved many elaborate studies and modeling. Each new study goes deeper into the same issue. We cannot follow such a train of experiments, as they quickly get complicated. We instead touch on some of the core insights. A prediction of the positional image matching hypothesis is that bees either need huge numbers of stored images to account for all the directions they might approach a target from, or they need to limit the directional approaches used. It has been shown that they attempt to approach close targets from consistent directions (Collett and Baron, 1994). Another issue relates to how much discord can exist between the stored image and the current view in order for image matching to work. Much modeling, and some experimental data, have explored this, with the concept of the catchment area (the area over which a given snapshot is useful) being the result (Zeil et al., 2003). Finally, motion parallax—how objects change in size and relative position to one another as the animal moves relative to them—has been shown to be a useful source of information (Zeil, 1993, Lehrer and Collett, 1994). It is thought to provide input important for landing, for example.

THE SEARCH FOR A BEE COGNITIVE MAP

A mental map refers to stored relationships between landmarks in the environment (Cheeseman et al., 2014b). This often means the distances and directions from one known place to another. In vertebrates, a cognitive map has been shown to exist, and some of its neuronal basis is known (Moser et al., 2008). "Place cells" in the hippocampus of the rat, for example, fire differently based on where the rat is in a room. For insects, a cognitive map has not yet been shown, although place cells have been shown in the cockroach using an experimental approach based on the rodent studies (Mizunami et al., 1998). In bees, there is much disagreement as to whether a cognitive map is present. Menzel and collaborators have conducted several studies over the last 15 years or so that they argue convincingly demonstrate a cognitive map.

Early work on bee navigation, as opposed to ant navigation, was compromised by the difficulty of tracking their flight paths accurately over long distances. An ant displaced from a known position can easily be followed back to her nest by a walking observer (Wehner and Srinivasan, 2003). A bee displaced can only be watched as she flies away. Because the direction of the bee's disappearance vector matched the homeward path integration expectation, it was assumed that the distance component also matched the path integration expectation. The more recent use of harmonic radar technology enabled accurate recording of bee flights. Using this technology, Menzel et al. (2005) showed that when displaced from a feeder to a novel place a bee flies for only a short time along the original homeward vector. She quickly realizes that she is off course and begins a search. This appears to last until she finds a known landmark, at which point she makes a bee line home. There are two interpretations of this result. Either the bees have memorized homeward vectors for every landmark they know (Collett et al., 1998, Cruse and Wehner, 2011), or they have a map sense that allows them to set a course for home from any known place.

Menzel and coauthors then began to test whether the bees have the ability to set novel routes through space. The finding of such novel routes is often the telltale sign of a map sense. They made use of the fact that bees make context-specific use of the waggle dance. In short, bees use the waggle dance either to determine when a food source that is known to them is active again (in which case they return to this known site) or to find a new site (von Frisch, 1967). Essentially, if a bee is advertising clover, for example, then a recruit who knows where another site of clover is may go there rather than to the place being advertised. This is likely adaptive because (1) those old sites may be active again and (2) it is sometimes difficult to find new flower patches using the waggle dance. Menzel et al. (2011) trained bees to a feeder (without odor) a set distance from the hive, then turned it off. They then trained other bees to a new feeder in a different direction but at the same distance. The bees from the turned-off feeder then followed dances for the new feeder. As expected, some dance followers (those that followed fewer than five runs) flew to their old site rather than the new site. What is intriguing is that the bees that returned to their old site, which was dry, then flew directly from the old feeder to the new feeder site, a novel path. There are again two interpretations of this result. First, although the path is novel, it is not beyond the bee's ability, in principle, to calculate using vectors. This is because both places (the old and the new site) are connected to the hive. The path between them thus closes a triangle, and it can be calculated mathematically. Second, again, the bees could have used their map sense. It would be telling if clock-shifted bees (see the next paragraph) could still accomplish what was shown in this study.

A study by Cheeseman et al. (2014b) is seen by the Menzel group as definitive, as it directly tests the two hypotheses for what explains the previous results. If the bees are using vector integration to find novel paths, then this can only work with a functional sun compass. This is because the result of these vector calculations is a vector based on the sun's azimuthal position. Hence, they used anesthesia, which is known to "clock shift" bees (Cheeseman et al., 2012), that is, to disrupt the clocks of bees taking novel routes home. They used the experimental context from Menzel et al. (2005), moving bees from a feeder to a novel place and recording the trip home. The experiment was done in a context in which the authors argued that the skyline provided insufficient information for navigation. The control bees (those not clock shifted) when released at a novel place flew, as per usual, in the direction specified by the homeward vector from the feeder (inferred based on the sun compass), but when they realized they were lost they searched for a time before flying straight home. The clock-shifted bees initially flew in a different direction (showing their sense of time was off), but after a search they also flew directly home in the same amount of time as the control bees. Cheeseman et al. (2014b) interpret the results to mean that the bees used local landmarks and their map sense to navigate the novel route, as they had no working sun compass and could not have combined stored vectors to find their way home.

CRITICISM OF THE MAP WORK

Cheung et al. (2014) argued that Cheeseman et al. (2014b) did not control for the use of alignment matching to the panorama and that the clock-shifting mechanism they used does not work. Cheeseman et al. (2014a) responded that they had considered the panorama, which they argued did not contain enough information for navigation. They then argued that their clock-shifting mechanism was not new to their study and that it works. We do not go into great detail because, frankly, there is not a lot of detail in the back-and-forth. No discussion of how accurate image matching would be in the context is given, for example, just that they could have used it. None of these papers refers to the magnetic sense, which might provide a rough direction, or the idea of two path integrators and the possibility of using the path integration for distance but not direction when making novel routes. Further, the next step, showing that they can plot novel paths to locations other than home (without a functional clock) has not yet been done. Thus, the question of whether bees have a map sense is still unresolved.

Circadian Rhythm

Biological clocks are central to many activities (Moore et al., 1998). Bees, like most animals, have basic daily sleep and activity rhythms, and engage in several periodic behaviors. Queens and drones, for example, take their mating flights at the same time (drones a little earlier), and workers take their orientation flights each afternoon. The clock is also used to calibrate the sun compass, as we discussed earlier. Beyond these basic uses, bees also make extensive use of their clock in food collection. A forager can remember nine different times and locations, for example, associated with flower patches (Koltermann, 1971). The bees thus have a derived timekeeping sense, and a considerable body of work has explored it.

Phenotypic Studies of the Clock

Early work demonstrated that bees have circadian rhythm at both the individual and colony levels (Medugorac and Lindauer, 1967, Southwick and Moritz, 1987, Frisch and Koeniger, 1994, Moritz and Kryger, 1994, Moore, 2001). This pertains to activity levels and to the timing of sleep (Kaiser and Steiner-Kaiser, 1983, Klein and Seeley, 2007, Eban-Rothschild and Bloch, 2008, Klein et al., 2008). The time when bees develop a circadian rhythm has also been the focus of study. Most of the work referenced above showed no rhythm for cell cleaners or nurses, moderate but clear circadian rhythm for middle-age bees, and strong rhythm for foragers.

Bloch and collaborators have followed up on the early work. They have shown, for example, that reverted nurses (foragers forced to return to nursing) also show no circadian rhythm (Bloch and Robinson, 2001), thus suggesting that the absence of rhythm is adaptive. They also showed that the treatments (JH application, etc.) that trigger precocious foraging do not regulate circadian rhythm (Bloch et al., 2002, Bloch and Meshi, 2007). With respect to the lack of a rhythm in nurses, Shemesh et al. (2010) showed that the brood is the likely source of the social cues damping circadian rhythm. Young bees caged with brood in a nest show no circadian rhythm, while young bees caged in the nest without brood do (figure 7.5). They further demonstrated that social zeitgebers are dominant to the light-dark (LD) cycle. When the two zeitgebers are put out of sync, bees entrain to the social cues over the LD cycle (Fuchikawa et al., 2016). They have also followed up on earlier work showing that the social cues used to coordinate circadian rhythm are likely substrate vibrations or pheromones (Siehler and Bloch, 2020).

Mechanistic Studies of Circadian Rhythm—Cyclical Gene Expression

Bloch and coauthors have also pioneered the study of the molecular basis of circadian rhythm in bees. Early work explored mainly gene expression, while more recent work has focused on outlining the bee clock machinery at both the molecular and neuronal levels (covered in the previous chapter). Several early

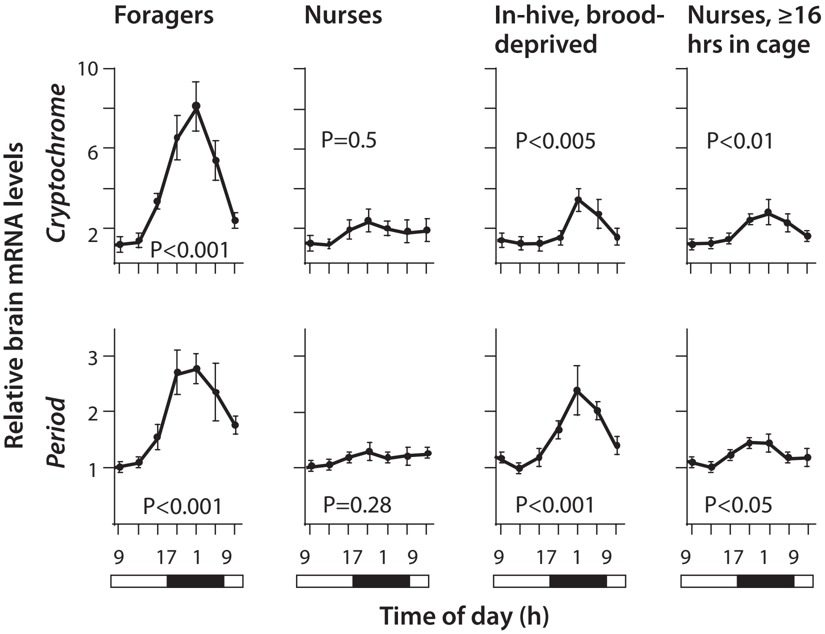


FIGURE 7.5. Period cycles in the brains of nurse-age bees in the hive without access to brood, as it does in caged young bees, suggesting brood is the source of the inhibition in period cycling (*from Shemesh et al., 2010*).

studies showed that the period gene, a core component of the central clock, cycles in foragers but not in nurses (Toma et al., 2000, Bloch et al., 2001). This suggested that the nurse clock was inactive. However, an alternative explanation—that the clock was running, but was not connected to its normal output channels—was also suggested by data. A transcriptomic study, for example, showed 160 genes cycling in nurses, a smaller number than in foragers (541) but enough to suggest a functional clock (Rodriguez-Zas et al., 2012). There were also data showing that nurses removed from the colony quickly become rhythmic with an entrainment based on the LD cycle, suggesting their clock had been entrained in the nest (Shemesh et al., 2007). The answer to these conflicting results came recently when Fuchikawa et al. (2017) used antibodies to label the bee clock neurons. They found that nurse clock neurons cycle (figure 7.6). The earlier results, based on whole-brain mRNA extracts, were misleading as period is expressed in glial cells across the brain, and it is these cells that cycle in foragers but not nurses. This work suggests that the output of the clock in nurses is damped by social cues, possibly those controlling

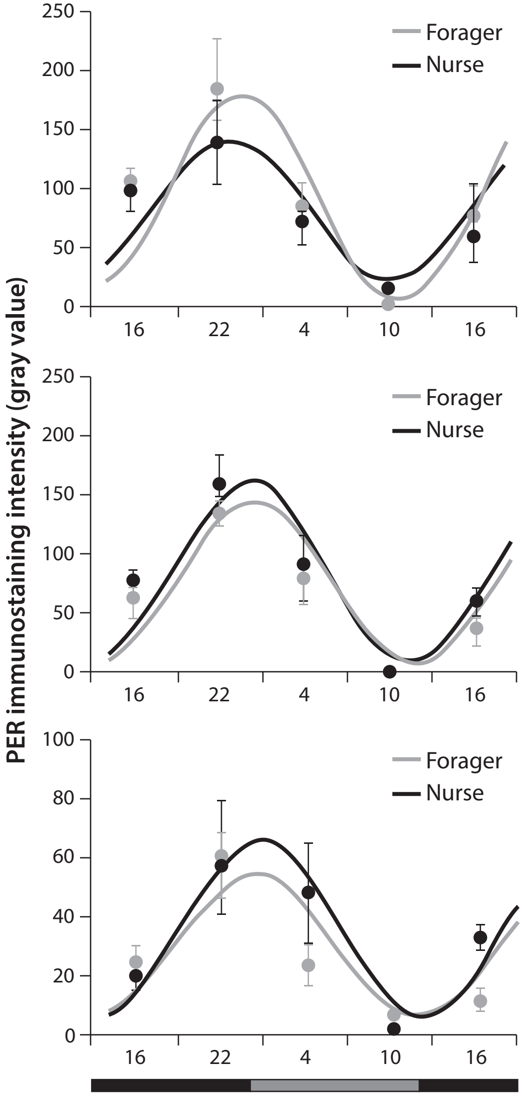


FIGURE 7.6. Similar rhythmic oscillations in period staining in the clock neurons of nurses and foragers. Results are for the DLN (top), the LN_1 cluster (middle), and the LN_2 cluster (bottom) (*from Fuchikawa et al., 2017*).

the transition into the nursing caste. It also suggests glial cells are important regulators of the output of the central clock to other brain centers.

The Magnetic Sense

It is clear that bees can sense the earth's magnetic field (Gould, 1980, Gould et al., 1980). Early work by Lindauer and Martin (1968), for example, showed that the waggle dance's orientation is modified by the earth's magnetic field. Magnetic effects on the orientation of comb building were also shown (Gould, 1980). Bees can also learn to associate magnetic fields with rewards using PER (Walker and Bitterman, 1985, 1989a, 1989b, 1989c, Liang et al., 2016). Gould et al. (1978) argued that bees sense magnetic fields with iron particles in their abdomens, which was later confirmed (Hsu and Li, 1994, Desoil et al., 2004). Hsu et al. (2007) showed that changes to the magnetic field cause the iron particles to expand, causing stress on the cytoskeleton, which triggers increased cellular levels of CA^{2+}, a potential mechanism for how the sensor works.

8

Reproduction

A bee colony can be viewed either as a superorganism (one individual) or as a society composed of individual bees. There are advantages to both approaches, depending on the context. In the present case, there is much to gain by taking both approaches. This is because the colony clearly reproduces as a colony, while individuals have interesting and derived (relative to solitary bees) patterns of reproductive physiology. We begin with the colony-level approach and conclude with individual-oriented physiological studies.

Colony-Level Reproduction

Swarming

Honey bee queens cannot take care of themselves at any life history stage, meaning that normal honey bee reproduction is fundamentally a group process via fission of the colony. While we have a thorough understanding of the relevant natural history, our understanding of the mechanistic causes of swarming remains weak. There are some topics within this subject, however, on which there has been considerable progress in the last couple of decades. These are queen duels, house hunting, and pheromonal control of queen and worker reproductive biology. Of these topics, we cover queen duels in this chapter and only touch on the others, as they are better covered elsewhere.

NATURAL HISTORY OF SWARMING

Swarming is a seasonal process, although it can happen any time of year (reviewed in Winston, 1991). Spring is the main period, but there is often a small second burst of swarming in the fall (figure 8.1). Swarming is often thought to

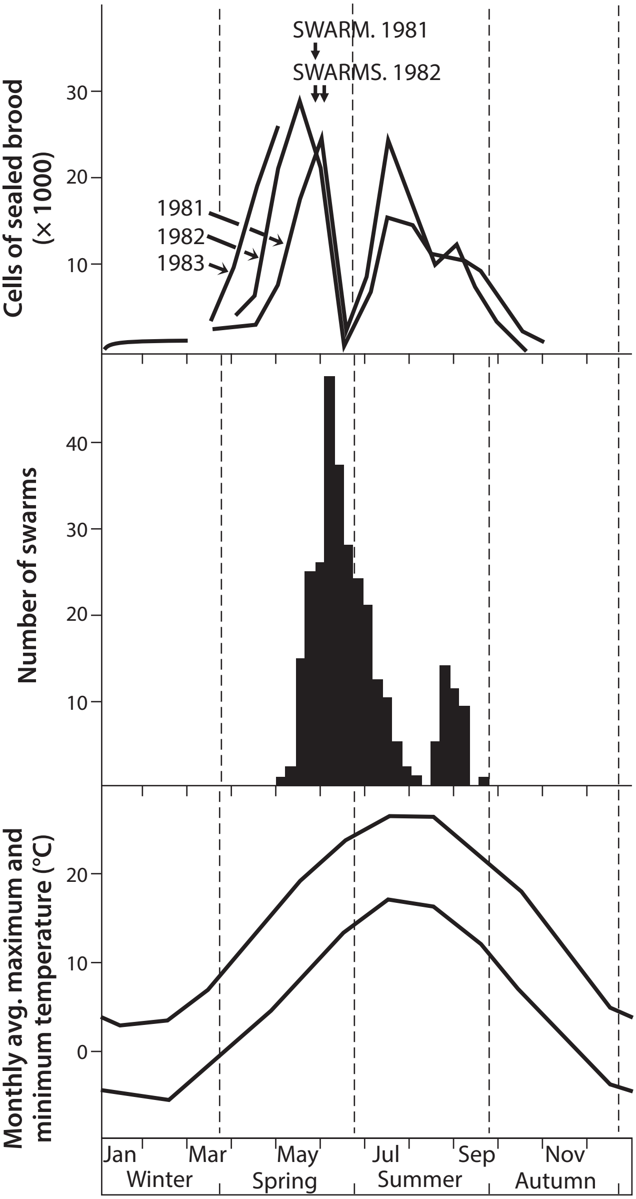


FIGURE 8.1. Seasonality of swarming. The strong spring peak in swarming is associated with large numbers of sealed brood cells (*from Seeley and Visscher, 1985*).

begin when the queen lays eggs in queen cups, typically 15–25 (Simpson, 1959, Caron, 1979). As the new queens begin their development, the workers prepare the old queen for swarming (Allen, 1956). A laying queen is too heavy for sustained flight, so she must shrink down. This process is associated with reduced feeding and the use of the shaking signal (Allen, 1958). Queens receive hundreds of shaking signals each day in the buildup to swarming (Fletcher, 1975, Pierce et al., 2007). Whether this serves a physiological or behavioral purpose (or both) is not understood. Traditionally, it has been thought that this signal causes the queen to move quickly about the nest so she loses weight, but given the data by Schneider et al. (2004c) showing changes in hormonal (JH) titer in workers after receiving this signal, this process may be more sophisticated than previously thought. Shaking signals could change queen physiology, leading to higher metabolism, or some other effect, causing priming of the queen for flight. Hopefully, future work will investigate this possibility. A few days before the swarm leaves, foraging also begins to slow down and scouting for new nests begins (Lindauer, 1955, Rangel and Seeley, 2008, Seeley, 2010, Rangel et al., 2010b). Workers begin to gorge themselves on honey, an adaptation for carrying as much food with them as possible to the new nest (Combs, 1972). Swarming proper typically happens a day or two after the first queen cups are capped, although it can be delayed for bad weather. When the bees are ready to swarm, workers begin to make buzz runs through the nest, which culminates in a large burst of bees emerging (Esch, 1967, Rangel and Seeley, 2008). The queen is driven out by the workers, and a cluster of bees forms around the queen on a nearby branch (plate 8). From this place, house hunting begins (Seeley, 2010). This first swarm, called the primary swarm, contains most of the workers in the hive (70%–80%) and is biased toward the younger workers (Butler, 1940, Winston and Otis, 1978, Rangel and Seeley, 2012). Meanwhile, back in the nest, the colony is temporarily queenless until a virgin queen emerges. At this point, a colony-level assessment is made regarding whether an afterswarm will take place. This is a second swarming event that is associated with the colony still being strong enough to split again. Some evidence suggests that the area of closed brood might be the relevant source of information (Winston, 1980, Winston et al., 1980). If an afterswarm is produced, then the workers protect the virgin queens still in their queen cups from the first virgin to emerge (Gilley and Tarpy, 2005). This involves much harassment of this queen (via biting, clamping, chasing, etc.) until swarming can happen. Afterswarms are much smaller than primary swarms (typically a couple thousand to about 10,000 bees, although this is quite variable). At this point, additional afterswarms may happen by repeating these behaviors, or the colony may stop swarming. If the colony stops swarming, then the next virgin queen to emerge chews holes in the cells of virgin queens still in their cups and stings them to death. If more than one virgin manages to escape during this often messy process, then the workers allow the queens to fight until all but one is killed. The new queen then mates and reproduction is complete. Because swarming occurs when the colony is nearly full of capped brood, the colony rapidly recovers from the loss of workers.

CAUSES OF SWARMING

Much work has gone into exploring the causes of swarming, mainly in the older literature. Our understanding of this process is far from complete, however, for two reasons. First, swarming is complex and is dependent on many variables, making study of the subject inherently difficult. Second, most of the work on swarming has occurred in North America and the honey bee is not native to this region. The mismatch between the seasonality one finds in the introduced range and that in the native range likely makes for both maladaptive behavior on the part of the bees and difficult-to-interpret experimental results. This is true even for European bees (of different subspecies) in North America, as significant genetically based variation in life history has been found even within the country of France (reviewed at the end of this section).

It is useful to break the causes of swarming into two categories: internal and external. Internal refers to the state of the colony, in other words, whether the colony is strong enough to reproduce (Rangel and Seeley, 2012). External refers to the environmental conditions under which selection has determined reproduction to be the most advantageous. Essentially, the time must be right and the colony must be in the correct state. Given that colonies are known to swarm year-round at low levels, bees will clearly swarm even if the time is not right, but they are biased to swarm when conditions are best.

With respect to internal factors, correlational data suggest that important variables may be colony size, percentage of closed brood cells, congestion in the nest and its effect on pheromonal dispersion, and the age ratio of the bees (Grozinger et al., 2014). Studies in which these effects are varied, however, do not reliably lead to swarming (Allen, 1956, Simpson, 1957, 1958, Winston, 1980). The clearest study is one by Lensky and Slabezki (1981), showing that limiting levels of either queen mandibular gland pheromone or queen tarsal gland pheromone alone do not lead to queen cup production, but that limiting both does. This pattern of queen signaling is consistent with a healthy queen in a congested nest. In other words, crowding limits the healthy queen from spreading her footprint pheromone around, which is a good cue for the workers to know it is time to swarm.

External factors have received less study, but photoperiod, food availability, and temperature are widely associated with the onset of reproduction across the insects, and these are likely culprits here as well. Kefuss (1978), for example, showed that photoperiod affects brood rearing, and Seeley (1985a) showed the importance of timing the start of brood rearing so swarming happens prior to peak food availability. Another key study, on a separate but related topic, showed that peak brood rearing, which precedes swarming, varies in time between two regions of France (Louveaux, 1973). A cross-fostering experiment showed that transplanted colonies maintained their temporal pattern, suggesting a genetic basis for this trait. This suggests that studies of subspecies outside their native range may lead to maladaptive behavior on the part of the bees. This might explain perplexing results, such as the production of afterswarms too late in the year. Under optimal conditions, even a small afterswarm can be successful, but those optimal conditions might not be present in the introduced range or may not be linked to the same external stimuli. Future work might benefit from conducting studies of swarming with wild-caught bees in their native range.

Queen Duels

Queen duels have been described by Gilley and Tarpy in a series of careful ethological studies (figure 8.2; Tarpy and Gilley, 2004, Tarpy et al., 2004, Gilley and Tarpy, 2005). The fights are very chaotic affairs with the queens being harassed by workers who shake and pin them down occasionally. In addition to biting and stinging, which is fatal and ends the fights, the queens also spray fecal matter onto one another. It has been suggested that this is a tactic to immobilize a rival (Tarpy and Fletcher, 2003). In addition to the kin selection hypothesis for worker participation (covered in chapter 9), another study looked at whether workers favor higher-quality queens, that is, target their aggression to low-quality queens. They do not appear to do this, even though the variation in quality in the study was much greater than is typical in nature (Tarpy et al., 2016). The duels appear to be a fair fight to the death, and they

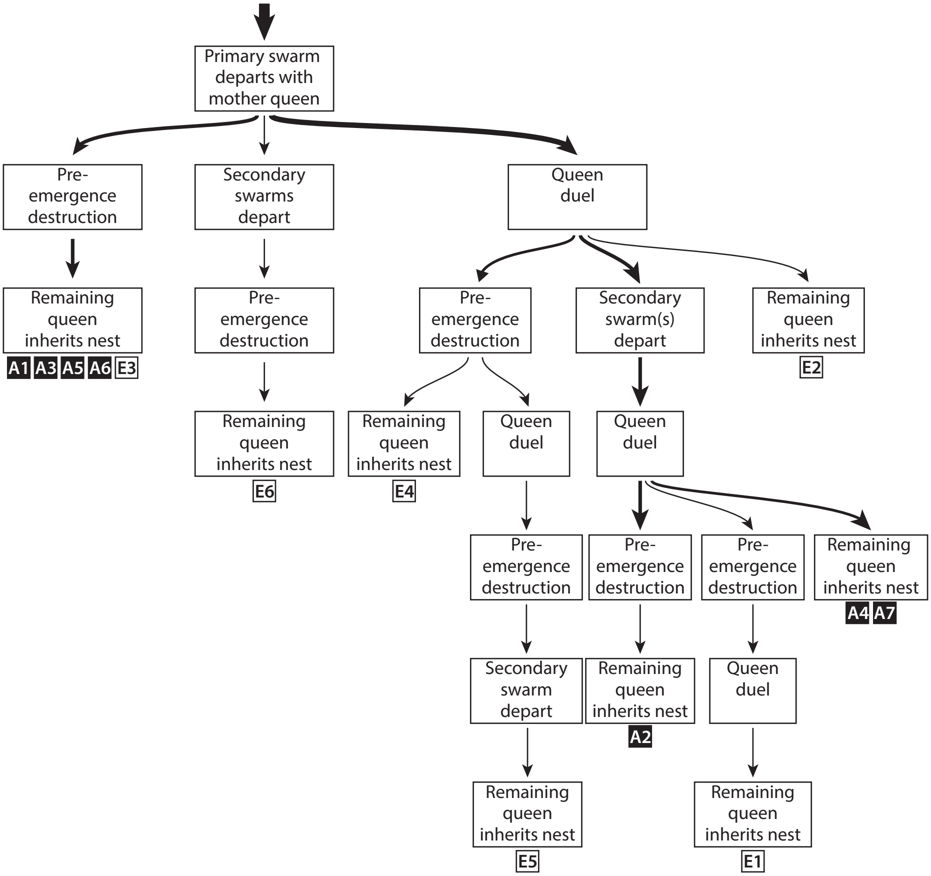

Primary swarm departs with mother queen
Pre-emergence destruction
Secondary swarms depart
Queen duel
Remaining queen inherits nest
A1 A3 A5 A6 E3
Pre-emergence destruction
Pre-emergence destruction
Secondary swarm(s) depart
Remaining queen inherits nest
E2
Remaining queen inherits nest
E6
Remaining queen inherits nest
E4
Queen duel
Queen duel
Pre-emergence destruction
Pre-emergence destruction
Pre-emergence destruction
Remaining queen inherits nest
A4 A7
Secondary swarm depart
Remaining queen inherits nest
A2
Queen duel
Remaining queen inherits nest
E5
Remaining queen inherits nest
E1

FIGURE 8.2. Flowchart showing the chain of events that occurred in 13 colonies after the mother queen departed with the primary swarm. Queen duels occurred in about half the cases and often occur in colonies that issue afterswarms (*redrawn from Gilley and Tarpy, 2005*).

presumably lead to the higher-quality individual winning on average (Long et al., 2017).

Drone Production

Drone rearing peaks a little over a month before swarming, so that the drone population peaks around the time of the swarming peak (Allen, 1963, Page, 1981, Seeley, 2019). The seasonal peak is easy to interpret given that the goal of the drones is to mate, and their development time (from egg until ready to conduct mating flights) is about 35 days (Metz and Tarpy, 2019). Only colonies of sufficient size produce drones, and colonies avoid storing honey in drone comb early in the season to avoid interference with drone production (Smith et al., 2014, 2015). The smaller numbers of drones produced later in the year are presumably expected to mate with queens produced for supersedure or for those colonies swarming at atypical times.

Interestingly, a honey bee colony has a 1:1 sex ratio between investment in males and females, if you consider the full swarm to be the investment in the female sex, which makes sense (Hamilton, 1975, Seeley, 2019). Thus, many thousands of drones are produced by a colony of typical size.

Supersedure and Emergency Queen Rearing

Supersedure refers to the colony's ability to replace a failing queen, while emergency queen rearing refers to the ability to produce a new queen when the old one suddenly dies. The two processes likely have some mechanisms in common, in that they depend on queen fertility signaling. Two classic studies showed that queens are replaced when they are old or are restricted in their movement (Butler, 1954, 1957). When a colony was divided, for example, with a screen separating the workers in the two halves, the workers housed with the queen did not produce supersedure cells, but those without direct contact did. This suggests some pheromonal control of supersedure. The pheromones were unidentified. A speculative guess for where to look might focus on the tarsal and Dufour's glands (Lensky and Slabezki, 1981, Katzav-Gozansky et al., 1997, 2001). However, pheromones alone cannot control this process. Physically damaged queens and drone layers can also be replaced (reviewed in Winston, 1991). It is therefore likely that several pheromones along with other cues are used to inform the workers' decisions regarding supersedure. Overall, supersedure is a reasonably common event, with 20% of colonies thought to requeen themselves each year (Allen, 1965). The contemporary number in beekeeping operations may be higher.

Although supersedure and emergency queen rearing are similar, there are some differences. When an old queen is replaced, normal queen cups are used to rear the new queen. This is similar to what occurs in swarming, although fewer queens are reared. In emergency queen rearing, the queen is missing, so she cannot lay eggs in prepared queen cups. The workers, rather, expand worker cells containing young larvae into queen cells, which jut out of the comb at a downward angle. These can be anywhere on the comb, as opposed to normal queen cups, which hang off the bottom. Emergency queen rearing is also a major cost to the colony, since no new brood are being reared until the new queen mates and begins laying eggs, which takes well over a month. This causes a sharp drop in population size and is a major source of mortality. Supersedure is less costly, as the old queen is often not killed until the new queen mates (Butler, 1957).

Queenless Colonies

When colonies lose their queen and cannot rear a replacement, some of the workers begin to lay unfertilized eggs, which develop into males (Punnett and Winston, 1983, Page and Robinson, 1994). This process is often incorrectly described, even in the primary literature, as "when the queen is lost, the workers begin to lay male eggs," which suggests that cooperation breaks down and the bees begin to reproduce as individuals. This is an oversimplification. Another important point to make regarding reproduction in queenless nests is that a honey bee colony typically has eggs and young larvae. Queenless colonies should be rare, therefore, but instead they seem common, at least in beekeeping operations. One explanation for the cause of high levels of queenlessness in apiaries may be related to what happens when colonies try to requeen themselves in this unnatural context. A queen returning from her mating flight might drift into another queenright nest and be killed (something highly unlikely in nature since colonies are so far apart). This is speculation, but it would be interesting to have data from wild colonies on the prevalence of queenless nests.

An overview of what occurs in a queenless nest is that the queen is lost, replacement measures fail, and a minority of workers activate their ovaries to lay drone eggs (Sakagami, 1954, Page and Erickson, 1988, Vanderblom and Verkade, 1991, Makert et al., 2006). A large batch of drone eggs is then laid, and pheromones from the developing larvae inhibit more workers from activating their ovaries. While this is occurring, most of the workers continue to work and the colony lives on until the workforce slowly dies out. Such colonies are easily identified by the experienced beekeeper because they produce a characteristic weak buzzing sound when opened that is clearly distinct from that of a normal hive. The cause or function of this is unknown. There is another discussion of queenless nests in chapter 9 regarding the question of nepotistic conflict.

Individual-Level Approaches to Reproductive Physiology—Honey Bee Mating

Honey bees mate in what are called drone congregation areas (DCAs). These are locations in which numerous males congregate and await the arrival of virgin females (Gary, 1962, 1966b). This practice is not unique to honey bees but is rather common in bees (social or solitary). The primary adaptive benefit seems to be the prevention of inbreeding since both queens and males travel considerable distances (several kilometers) from their nests and visit multiple DCAs. Baudry et al. (1998), for example, found that a single DCA contained drones from 240 different colonies. Much early work on DCAs involved descriptive work, such as how high the sites are up in the air, how far away from the nest, how many drones are present, and so forth. We omit much of this detail here and describe the basics of this behavior. The reader is referred to Winston (1991) for the quantitative details.

Considerable work has gone into trying to determine what factors are used by the bees to identify DCAs. The two pioneers of this topic, Ruttner (1956a) and Gary (1966b), both found that DCAs are consistent in location from year to year. Ruttner, in particular, found that they tended to occur in areas with similar features, leading to a search for the cues used to find them. A more recent study made similar discoveries (Galindo-Cardona et al., 2012). However, the environmental features associated with DCAs are not the same across locales (reviewed in Winston, 1991). This may be because DCA location depends more on technical features of the bee's sensory system than on objects in the environment. The results of such sensory biases could manifest in confusing ways across different habitat types. Another popular idea is that DCAs could be found using the bee's poorly understood magnetic sense (Gould et al., 1978, Liang et al., 2016).

What happens at DCAs is relatively well known based on studies in which tethered queens, or lures impregnated with queen pheromone, are flown through them (Gary, 1962, Strang, 1970). In a nutshell, the queen flies through the DCA with a comet-like formation of drones in pursuit. Drones approach queens from below, and mating involves the male grasping the queen with his legs before inserting his endophallus and doing a back flip, which results in the breaking off of his endophallus and the explosive discharge of semen and seminal fluid into the queen's reproductive tract (figure 8.3). The seminal secretions form a mating plug, the function of which is still open to debate. It may facilitate cementing the in-flight connection between male and female (Koeniger,

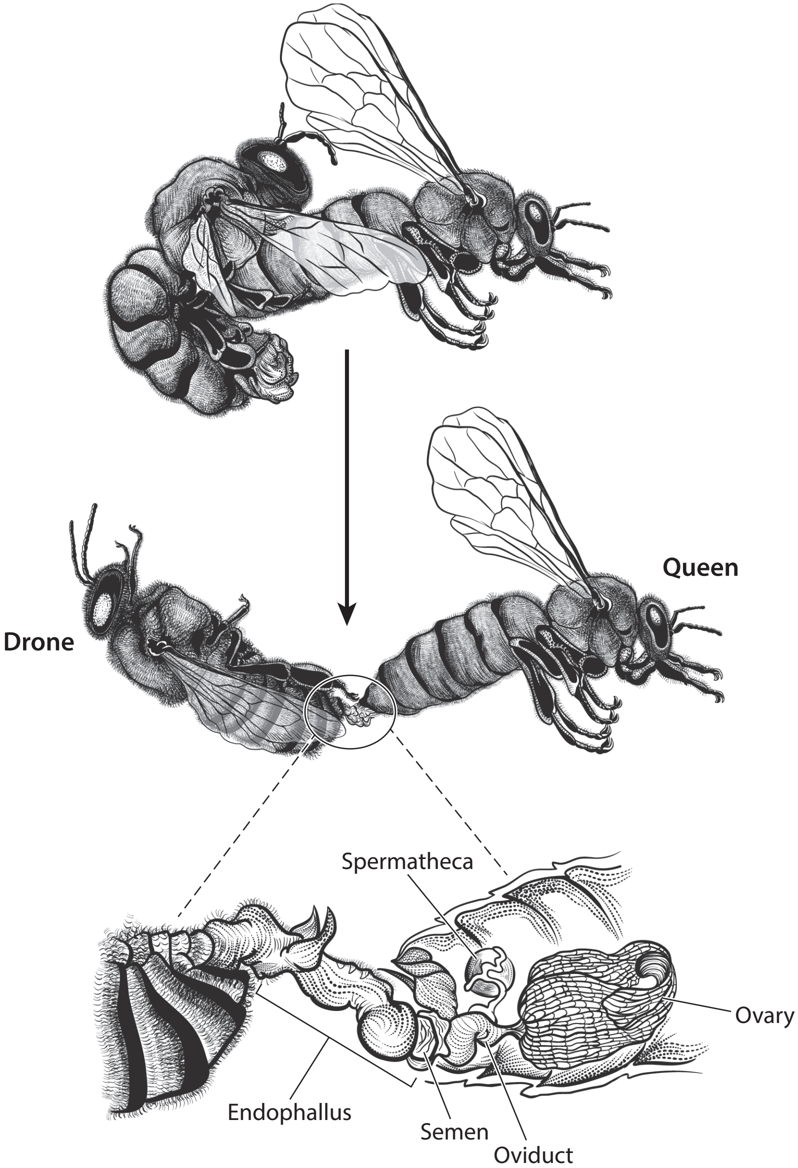

Queen
Drone
Spermatheca
Ovary
Endophallus
Semen
Oviduct

FIGURE 8.3. Sequence of acts in honey bee midflight mating and a diagram of the male and female internal sexual organs during mating (*redrawn based on Winston, 1991*).

1986, 1990). It could also prevent semen from escaping the queen's reproductive tract (Bishop, 1920, Woyciechowski et al., 1994). It also might be a standard insect mating plug, which is ineffective based on selection to overcome it from the female side. In either case, the mating plug does not seem to interfere with future mating, as drones are able to brush it aside. There is some suggestion, however, that multiple mating plugs may prevent future mating (Woyke, 2016). An interesting idea is that the mating sign may be an example of honest signaling (cooperation) between drones (Koeniger, 1990), the idea here being that a queen who has mated is of higher quality than one who has not and the plug advertises this fact. Interestingly, the mating sign is unique to the cavity nesting *Apis*, as the species that live in the open do not produce copious seminal secretions (Colonello and Hartfelder, 2005).

Most queens take multiple trips to DCAs, usually two but sometimes more, and mate with 12–15 males on average per mating flight (Ruttner, 1956a, Fuchs and Moritz, 1999, Tarpy and Page, 2000, 2001, Tarpy and Nielsen, 2002). It is not clear what triggers cessation of mating, but perhaps the best-supported hypothesis is that the filling of the oviducts leads to cessation of mating flights (Woyke, 1964, Koeniger and Koeniger, 2007, Richard et al., 2007, Nino et al., 2013b, Simone-Finstrom and Tarpy, 2018). When mating is complete, semen from all males is mixed together and stored for the lifetime of the queen in her spermatheca (Woyke and Ruttner, 1958, Woyke, 1964, Haberl and Tautz, 1999). She never mates again. Although mixing is not perfect, it is quite involved, and all patrilines are thus present in the queen's offspring in proportion to the volume of semen contributed by their father, which does not vary much (Franck et al., 2002, Schluns et al., 2004).

Evolution of Polyandry

As we mentioned previously, a queen honey bee mates with 12 males on average (Tarpy and Page, 2001). Such extreme polyandry is the norm for the genus *Apis* (Oldroyd and Wongsiri, 2006). Extreme polyandry, however, is not the norm in the social insects, although modest polyandry is not uncommon (Crozier and Fjerdingstad, 2001). Most social insects mate once and have a single queen. This is the case for most stingless bees, for example (Palmer et al., 2002). The adaptive basis of extreme polyandry is thus an interesting question. This is particularly so since it decreases the mean relatedness of workers and thus should be selected against from a kin selection perspective. There have been about a dozen hypotheses proposed to account for extreme polyandry

(Page, 1980, Sherman et al., 1988, Crozier and Fjerdingstad, 2001). We focus on the hypotheses that have received the most attention in honey bees.

Page proposed that extreme polyandry is an adaption to avoid the production of diploid males (Page, 1980, Kraus and Page, 1998). A queen who mates with a male at a DCA who shares a sex allele with her will have compromised brood viability, and the likelihood of this can be decreased with multiple mating. Page and coauthors explored this from a theoretical and experimental perspective and showed that this hypothesis can explain some degree of polyandry (Page, 1980, Page and Metcalf, 1982, Tarpy and Page, 2000, 2001). However, it does not seem to be sufficient to explain extreme polyandry (more than 10 matings). As an aside, how social insects avoid inbreeding, given their massive production of males and females (sometimes at the same time), is a poorly understood phenomenon. How queens avoid mating with their brothers is probably quite variable across the social insects, and many traits thought to be associated with other questions (like split sex ratios) may be associated with inbreeding avoidance. This is speculative, of course, but the topic is deserving of more study.

Most of the work on the adaptive basis of polyandry is centered on increasing genetic diversity within the colony. There are two forms of this hypothesis. Either genetic diversity is necessary for, or increases the effectiveness of, task allocation, or it makes the colony more resistant to parasites and pathogens (Sherman et al., 1988, 1998, Shykoff and Schmid-Hempel, 1991, Baer and Schmid-Hempel, 2001, 2003, Mattila and Seeley, 2007, 2014). We cover the task allocation hypothesis in chapter 13. Here we cover the immunity idea. Work in bumble bees showed that more genetically diverse colonies have lower pathogen loads and higher fitness (Baer and Schmid-Hempel, 2001, 2003). In honey bees, several studies used artificial insemination to create single- or multiple-patriline colonies, which could be challenged with parasites or pathogens. For chalkbrood and American foulbrood, multiple mating increased fitness and decreased variation across colonies in pathogen load (figure 8.4), while no effect was found for *Varroa destructor* resistance (Neumann and Mortiz, 2000, Tarpy, 2003, Tarpy and Seeley, 2006, Seeley and Tarpy, 2007). Studies have also looked at individual-level immune responses and showed that patrilines vary in their resistance to some pathogens (Palmer and Oldroyd, 2003, Simone-Finstrom et al., 2016). There is thus considerable support for the immunity hypothesis for extreme polyandry.

Overall, there is support for both the sex allele and the genetic diversity for pathogen resistance hypotheses. As these hypotheses are not mutually exclu-

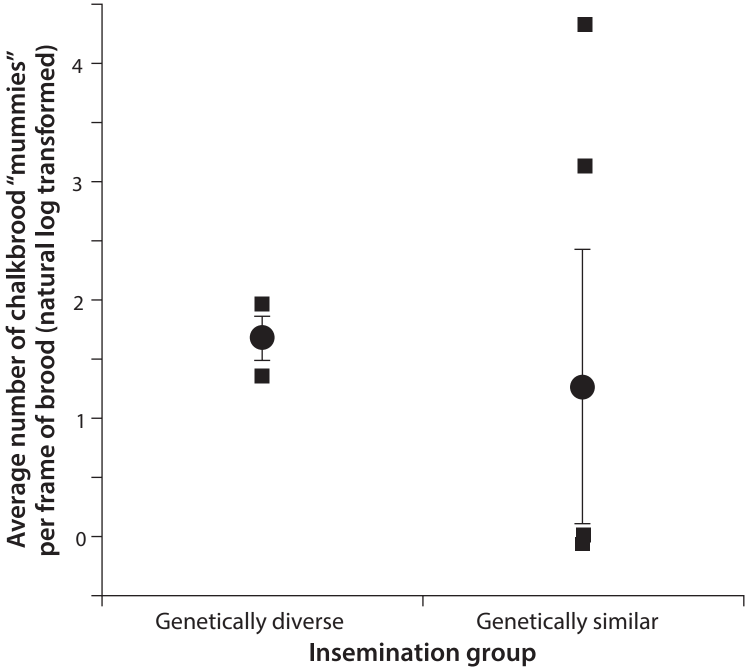


FIGURE 8.4. Genotypically diverse colonies have less variance in chalkbrood infections than do single-patriline colonies (*from Tarpy, 2003*).

sive, there is little reason not to suspect that at least both of these factors are playing some role in the evolution of polyandry.

Virgin Queen Behavior Postswarming

The basics of queen reproductive behavior, ignoring the complexities of swarming, are straightforward. The queen emerges and matures in the nest over five to ten days. She then takes mating flights (often two to three), and begins laying eggs a few days after mating (reviewed in Winston, 1991). Although this is simple in broad strokes, it is an involved process.

For the first few days of life, assuming swarming is over and competitor queens are dead, a virgin queen is largely ignored by the workers. She feeds herself and does little. By day six, she is reproductively mature, and a court of workers (her "retinue") surrounds her, licking and inspecting her abdomen (Ruttner, 1956a). She is now ready to begin orientation and mating flights. This is when the relevant natural history becomes intricate and poorly understood. The basic pattern is that workers appear to harass and/or communicate with the queen to get her to leave the nest (Hammann, 1958, Ruttner, 1956a, Lensky and Demter, 1985). The queen may initiate all of this by signaling her fertility to the workers (Ruttner, 1956a). The normal process seems to involve walking the queen to the entrance, but if she resists, which she sometimes does, she is forced. The communication process (cooperative or aggressive) builds in intensity to a crescendo, at which point the queen leaves on a mating or orientation flight. The workers wait for her return at the entrance and produce Nasonov pheromone to ensure she finds her way home. The papers describing these behaviors are not the clearest, and replication of this work is necessary.

Individual-Level Research on Honey Bee Reproduction

Mechanistic Studies of Queen Reproductive Physiology

The process of activating ovaries begins immediately after mating and is associated with an increase in the level of vitellogenin production in the fat body and its uptake from the hemolymph in the ovaries (Tanaka and Hartfelder, 2004, Kocher et al., 2008). Likewise, after mating, the pheromonal profile from the mandibular glands changes (activates) and queens become attractive to a retinue of workers, which is typical for a laying queen (Kocher et al., 2009). We cover this in more detail in chapter 14 on chemical signaling. Finally, Fahrbach et al. (1995) showed that changes occur in the queen's brain after mating; specifically, changes to the organization of the mushroom bodies and changes in dopaminergic signaling. The changes in mushroom body organization also occur in workers after they transition to foraging and in males prior to orientation flights (Withers et al., 1993, Fahrbach et al., 1997). At least some of these changes are thus likely associated with flight and orientation rather than caste-specific behavior or physiology.

For all these changes, it is unclear what endocrinological or epigenetic regulatory mechanisms control the process and how they interact with the process of mating upstream and the control of gene expression in thousands of genes downstream. This is due to a series of studies with conflicting results. Robinson et al. (1991) found that juvenile hormone (JH) is not associated with queen reproduction, but ecdysteroid (Ec) is higher in laying queens than in virgin queens. Yamazaki et al. (2011) found evidence for tissue-specific production and function of Ec in the ovaries and brain. They also confirmed some of the patterns found by Robinson et al. (1991). Wegener et al. (2013) found that Ec titer falls in queens after mating, again questioning past results. They also showed that JH is higher in virgin than in mated queens and increases in titer in caged queens not able to lay eggs. In sum, how JH and Ec, and perhaps other hormones, control queen reproductive physiology is unresolved.

A separate body of work has explored the changes in the several glands that are strongly associated with queen reproduction. Kocher et al. (2008), in a study of virgin, mated, and laying queens, showed that the activation of the ovaries, the activation of the mandibular glands, and the changes in the brain occur independently of one another, as they are temporally out of sync. They also identified a list of candidate genes that may be involved in this process. Nino et al. (2013b) in a similar study showed that insemination volume may trigger a cessation of mating flights, but substances in the seminal fluid may trigger the various physiological responses leading to laying and reproductive signaling. A second study by Nino et al. (2013a) showed that mandibular gland activation is dependent on many factors, including seminal fluid volume, physical manipulation of the genital tract, and seminal compounds, while Dufour's gland activation is dependent only on compounds in the seminal fluid.

Reproduction in Drones

The physiology and behavior of drones is understudied relative to workers and queens for rather obvious reasons. The interest in the honey bee stems from their social behavior and their economic utility, and drones contribute to neither. Nevertheless, there is some work on drone biology. It is important to start by pointing out that drones are highly specialized for their limited life history. They do little other than mate. Accordingly, they have highly elaborate physiological adaptations related to this function and are missing most structures not related to mating. The work that has been done on drone behavior and physiology thus primarily relates to questions such as how their sensory system is specialized for finding queens, how their accessory glands make mating plugs, how mating flights are regulated, and so forth.

NATURAL HISTORY OF DRONE REPRODUCTIVE BEHAVIOR

After emergence, drones stay in the brood zone area, where they are fed by nurses (Szolderits and Crailsheim, 1993). Around the age of six to eight days, drones move to the periphery of the nest and begin to take orientation flights

(Ohtani and Fukuda, 1977, Reyes et al., 2019). They reach reproductive maturity at about 12 days (Metz and Tarpy, 2019). Mating flights begin immediately upon reaching reproductive maturity in the summer, but in the spring they wait until 21 days of age to begin taking mating flights (Reyes et al., 2019). Drones take multiple mating flights per day (range of about three to five). Drones that successfully mate die soon after; otherwise, drones live about 20–30 days, depending on the season—longer in the spring, less in the summer (Fukuda and Ohtani, 1977, Reyes et al., 2019). Those drones still present in the hive in the fall are driven from the nest by the workers (Seeley and Mikheyev, 2003).

COMMON AGING PATHWAYS IN DRONES AND WORKERS

There is extensive overlap between the genetic bases of maturation in workers and drones (figure 8.5). As for workers, drones also show an increase in JH titer as they age, and application of JH to immature drones stimulates the commencement of mating flights (Tozetto et al., 1995, Giray and Robinson, 1996). At the genomics level, Zayed et al. (2012) compared young and old drone brain transcriptomes, using microarrays, to identify changes in expression across the genome. The take-home message from this study was the strong overlap in the list of differentially expressed genes between aging workers and drones. This could also be somewhat of an artifact given the use of a microarray probably containing mainly highly conserved genes; but even if many sex-specific genes exist in drones, it would not change the fact that the conserved genes show similar expression patterns in workers and drones.

MALE ACCESSORY GLANDS

Accessory glands in male insects often play major roles in reproductive biology. Well-known examples are the glands that produce the nuptial gifts in crickets and those that produce the sex peptides in *Drosophila melanogaster* associated with reproductive conflict. In the honey bee, Colonello and Hartfelder (2003) showed that the protein content of the accessory gland changes during drone development. A complex mixture of proteins characterizes early drone development, while a less rich complement of proteins is evident at reproductive maturity. This work was done with SDS page, which is not terribly sensitive, so future proteomics work, coupled with RNA seq, is required to validate this result. However, a clearer result was obtained in the same study for the endo-

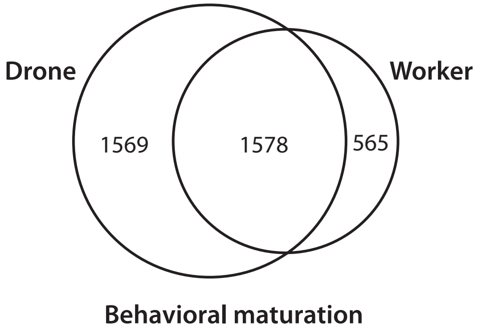


FIGURE 8.5. The majority of genes associated with maturation in workers also influence maturation in drones, suggesting common aging processes dominate development across the sexes (*from Zayed et al., 2012*).

crinological control of this pattern. Injection of ecdysteroid prevented the developmental changes in protein content, suggesting that Ec negatively controls development of the male accessory gland (Colonello and Hartfelder, 2003).

Colonello-Frattini and Hartfelder (2009) conducted a follow-up study at the transcriptomic level. A small number of expressed sequence tags (ESTs) were identified that were overexpressed in the accessory glands. Some of these genes were also expressed in worker venom glands, while many others did not have hits in the NCBI database, suggesting they are honey bee–specific novel genes. This is consistent with other studies in bees of glands, which are hot spots for rapid sequence evolution and novel genes (Jasper et al., 2015, Johnson, 2018). Another interesting result was that the gland had high levels of deformed wing virus, suggesting this virus could be an STD.

Reproduction in Workers

WORKER REPRODUCTION IN QUEENRIGHT COLONIES

A complex, still poorly understood mixture of pheromones inhibits ovary activation in workers in queenright colonies. We cover this in depth in chapter 14 on chemical ecology. In this section, we briefly discuss those workers who escape this inhibition. Overall, such bees are rare. Visscher showed that only 1 in 10,000 bees in a queenright nest has active ovaries, and these workers lay up to

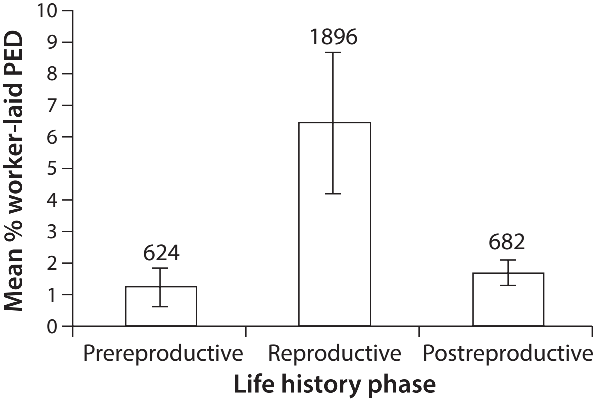


FIGURE 8.6. Worker egg laying increases during the reproductive phase of the colony life cycle (*from Holmes et al., 2013*).

7% of drone-destined eggs. However, policing of worker-laid eggs is efficient, and only 0.1% of males in a nest are not the queen's sons (Page and Erickson, 1988, Visscher, 1989). More recently, Holmes et al. (2013) challenged these conclusions with data suggesting worker reproduction may be more common during swarming. They found that workers produced about 4% of drones on average, and that worker egg laying and having activated ovaries was more common during swarming than had previously been suspected (figure 8.6). Swarming, of course, is a period in which queen pheromones are restricted, and the colony's great activity might make policing less of a critical need.

LAYING WORKER PHYSIOLOGY IN QUEENLESS COLONIES

Our understanding of the physiology of laying workers is limited. Early work showed that JH is not involved (Rutz et al., 1976, Robinson et al., 1991). It is not clear, however, whether Ec, the other major hormone, plays a role (Robinson et al., 1991, Hartfelder et al., 2002). Robinson et al. (1991) showed that ecdysteroid is higher in laying workers compared to nonlaying workers. Hartfelder et al. (2002) found no correlation between ecdysteroid titer and ovary activity in workers in queenless colonies. They also found no effect of queen presence on ecdysteroid titer. Trawinski and Fahrbach (2018), however, showed more recently that ecdysteroid levels vary in workers in response to queen mandibular gland pheromone (QMP) treatment. In summary, although there is considerable research on the role of Ec in worker reproduction (and behavior in general), no conclusions can yet be drawn, as the results—just as for queens—are contradictory. Our ability to address the issue may be prevented by methodological hurdles stemming from the difficulty of working with the low amounts of this compound in the bee body, which are likely tissue specific in function.

Other than the hormones just discussed, the other gene most commonly studied in the context of reproduction in insects is vitellogenin (Vg). Expression of this gene is extremely high in laying queens (Rangel et al., 2021). Several studies have shown that Vg expression goes up in queenless nests and laying workers have higher Vg titers than nonlaying workers (Engels, 1977, Engels and Fahrenho, 1974, Fluri et al., 1982, Nakaoka et al., 2008, Peso et al., 2016). Vg thus likely plays a similar role in worker reproduction as it does in queens (Koywiwattrakul and Sittipraneed, 2009).

Another approach to the physiology of laying workers has been to explore the role of biogenic amines. QMP, for example, is known to inhibit dopamine (DA) levels in the worker brain (Beggs et al., 2007), suggesting that queen loss would lead to an increase in this compound. This is the case, as activated ovaries are associated with elevated DA levels (Harris and Woodring, 1995, Sasaki and Nagao, 2001). Further, Dombroski et al. (2003) showed that feeding DA causes activation of ovaries in workers. Serotonin, another biogenic amine, is also associated with ovary activation (Peso et al., 2016).

There has been some work at the genetic/genomic level regarding laying workers. Preliminary work mainly searched for genes that differ between laying and nonlaying workers and explored common patterns of expression between laying workers and laying queens. Grozinger et al. (2007) found 221 differentially expressed genes between the brains of laying workers and queens. Niu et al. (2014) found 5000 genes that differ in expression in the ovaries of laying and nonlaying workers, while 1615 genes differ in expression between laying and virgin queens. About 800 genes were common to both laying workers and laying queens. These genes were distributed across many signaling pathways.

Some more recent work has delved deeper into the control of worker reproduction. Duncan et al. (2016) showed that notch signaling, a highly conserved pathway regulating growth and differentiation in many systems, is associated with the QMP-mediated downregulation of worker ovaries. Pharmacological inhibition of the notch pathway (both in the presence of QMP and in its absence) increased ovarian development in workers (figure 8.7).

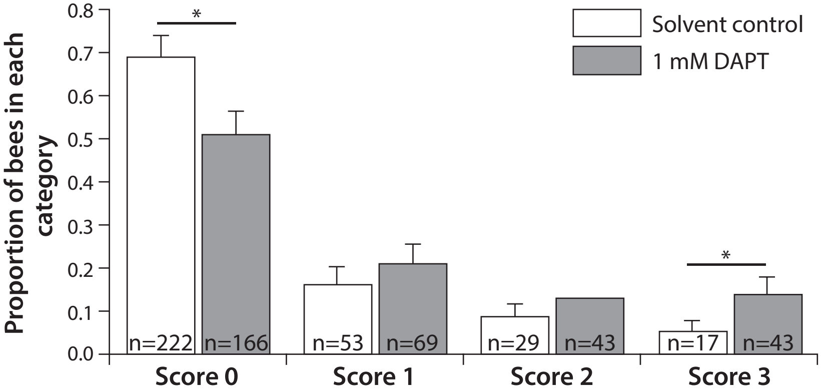


FIGURE 8.7. Inhibition of the notch pathway, using DAPT, increases ovarian activation in workers, measured with an activation score from zero (signifying inactive) to three (*from Duncan et al., 2016*).

Further, laying workers had decreased expression of the notch receptor in their ovaries relative to workers in queenright nests. A later study by the same group showed that chromatin structure is regulated by social signals (associated with queen presence) and genes associated with worker ovary activation are localized in arrays, making them easily regulated by histone modifications (Duncan et al., 2020). It is not clear if the localization of these genes to arrays is associated with social evolution or taxonomically restricted evolution across the Apidae. H3K27me3 marks, and notch signaling, were found to be associated with worker ovary activation, along with the epidermal growth factor receptor (*egfr*).

Parthenogenesis in Apis mellifera capensis

Parthenogenesis refers to reproduction without fertilization. Honey bees from all races, and many other eusocial Hymenoptera, have some ability to reproduce parthenogenetically, which is called thelytoky in haplodiploid organisms (Goudie and Oldroyd, 2018). Most virgin queens, for example, lay a small minority of diploid eggs (Mackensen, 1943, DeGrandi-Hoffman et al., 1991). This frequency varies, with some subpopulations laying diploid eggs more than others. Most work on thelytoky, however, in bees is conducted on the Cape honey bee from the tip of South Africa. This subspecies, *Apis mellifera capensis,* is characterized by widespread thelytoky in workers (Onions, 1912, Anderson, 1963, Hepburn and Crewe, 1991).

Verma and Ruttner (1983) showed that the mechanism underlying thelytoky in Cape honey bees involves a polar body fusing with the egg. They showed that the chosen polar body always contains an alternate sex allele. Moritz and Haberl (1994) showed that recombination is suppressed during the process. The genetic basis of thelytoky has been the focus of much recent work but is still unclear. Early work suggested the trait is under the control of a single recessive allele (Lattorff et al., 2005). Jarosch et al. (2011) collected data suggesting that this allele is from a transcription factor homologous to the *D. melanogaster* gene, *gemini*. Chapman et al. (2015), however, showed that this allele is found in *A. m. scutellata* as well as *A m. intermissa* (another African subspecies lacking thelytoky). F1 progeny of crosses between *A. capensis* and *A. scutellata* were also shown to be capable of thelytoky, which would not be the case if it were controlled by a single recessive gene. A couple of association mapping studies have followed, each identifying a favorite allele argued to control thelytoky (Aumer et al., 2019, Yagound et al., 2020). Christmas et al. (2019a) have shown that the allele identified by Aumer et al. (2019) is unlikely to be the cause of thelytoky because the single nucleotide polymorphism (SNP) is common in other subspecies and missing in some capensis queens. Finally, Wallberg et al. (2016) identified many loci that have undergone recent selection in *A. m. capensis* and are candidates for the genetic basis of the many traits associated with laying workers. The key issue, of course, is that correlation is not causation. Until clear experimental studies demonstrating cause and effect are conducted, the question will remain unresolved.

With respect to the adaptive basis of thelytoky, there are a number of hypotheses. It may have evolved as a means of surviving queen loss. When the queen is lost in capensis, laying workers produce diploid eggs, which are then used to rear a new queen, as opposed to workers laying drones, leading to colony death. Another possibility is that the trait arose to enable social parasitism. Capensis workers will invade nests from other subspecies and eventually take them over (Neumann et al., 2002, Neumann and Moritz, 2002, Martin et al., 2002). Capensis workers in this context engage in queen pheromone mimicry and behave as pseudo queens (Crewe and Velthuis, 1980, Simon et al., 2001). Parasitized colonies produce more capensis workers but quickly die off, in a manner typical for nest parasites across the social insects. It could thus be the case that this subspecies of honey bees is in the midst of evolving into a nest parasite (Neumann and Moritz, 2002) or has reached some stable evolutionary equilibrium that balances normal colony reproduction, with higher than normal levels of worker reproduction, with nest parasitism.

Against this argument is the fact that, until recently (due to intervention by beekeepers), there was a stable boundary between the *A. scutella* and *A. capensis* populations. It is thus not clear how common social parasitism was before beekeepers began moving colonies around.

Anarchistic Bees

Oldroyd and colleagues have explored worker reproduction using a rare mutation leading to worker reproduction in queenright colonies. These so-called anarchistic workers are characterized by two traits: activated ovaries in the presence of a healthy queen and the production of an egg-marking pheromone that prevents policing of their eggs (Barron and Oldroyd, 2001). In nature, colonies with this trait are extremely rare, and the trait is perhaps best thought of as a simple genetic disease (Oldroyd et al., 1994b). Laying workers are further rare in wild-type colonies containing anarchistic bees (Montague and Oldroyd, 1998). Selection and inbreeding of colonies with this trait, however, allowed for the production of a line of bees that reliably has laying workers (Oldroyd et al., 1999, Oldroyd and Osborne, 1999). These colonies cannot survive without beekeeper care, in that their colony-level mechanisms for maintaining order are terribly broken, but these colonies are useful (with care) for exploring the genetic basis of worker reproduction.

Two main bodies of work have grown out of studies of the anarchistic line of bees. First, a traditional quantitative trail loci (QTL) study was conducted that identified four QTLs associated with the syndrome (Oxley et al., 2008). Several promising gene candidates were identified in these QTLs, and one—called anarchy—was shown to strongly predict ovarian state (Ronai et al., 2015). The second body of work has explored the pheromonal basis of the anarchy syndrome. For example, it was shown that QMP does not differ between anarchistic and wild-type bees (Hoover et al., 2005), suggesting that the response of the anarchistic bees to reproductive signals, rather than the production of queen pheromone, may underlie their behavior. Second, it was shown that the egg-marking pheromone of the anarchistic workers is different from that of the laying queen's egg-marking chemical signal, although it has similar (though weaker) effects (Martin et al., 2004).

9

Evolution

Evolution spans all levels of biological organization, so an evolutionary perspective pervades every topic covered in this book. The sensu stricto evolutionary biology topics—kin selection, population genetics, and quantitative genetics—are split between two chapters. Quantitative genetics is covered in chapter 5, while kin selection and population genetics are covered here, along with the evolutionary genetics of eusociality.

Kin Selection

Kin selection theory revolutionized the study of social insects by providing a sound theoretical basis for the evolution of altruism. For self-sacrificing behavior to evolve, the benefits of the act weighted by the relatedness coefficient between the two actors must outweigh the costs (Hamilton, 1963, 1964a, 1964b, Axelrod and Hamilton, 1981). This is succinctly represented mathematically as, $rb > c$, with r being the relatedness coefficient and b and c the benefits and costs associated with the act. Another way to think about kin selection is to use the related term *inclusive fitness* (Trivers and Hare, 1976, Grafen, 1985, Queller, 1989, Queller and Strassmann, 1998). In a nutshell, one cannot measure an organism's fitness by recording their individual reproductive output. Rather, one must add to that a measure of indirect fitness that reflects the reproduction of their relatives that is the result of their help.

Kin selection theory is soundly supported across the tree of life, and many important results pertaining to social insects have been found (Hughes et al., 2008). However, kin selection studies in honey bees largely report a lack of nepotistic conflict, contrary to predictions. Although reviews rarely discuss these results in detail, they should—because a preponderance of evidence is accumulating against some kin selection ideas (not kin selection in general).

In particular, it is often assumed that a colony reflects a balance between conflict and cooperation, and this is certainly true, but it is not the case that this balance must be anywhere near equal. For some highly derived species, like the honey bee, the data suggest the balance is shifted far toward favoring cooperation. This is not to say that kin selection is not the appropriate theoretical framework from whence to explore honey bee cooperative behavior, but rather that the emphasis on variation in the r variable has led to neglect in the study of how the b and c terms can drive highly derived cooperation. Further, it may be that a precondition for the evolution of the most derived superorganismal forms of eusociality is a working resolution of most nepotistic conflict (Korb and Heinze, 2004). This would suggest that colony-level selection is paramount, at least with respect to what have traditionally been called the "advanced" eusocial species (Seeley, 1989a, 1997).

Kin Selection Studies in Honey Bees

In a species as socially derived as honey bees, it is taken for granted that their cooperative behavior is ultimately adaptive due to kin selection. It is clear that extreme self-sacrifice, for example, such as sick individuals leaving the colony, is explained by kin selection (Rueppell et al., 2010). Most kin selection research on honey bees, however, focuses on relatedness asymmetries in the colony and how they lead to nepotistic conflicts of interest (either potential or realized). The goal is typically to understand how these conflicts are resolved, and in whose favor (Queller and Strassmann, 1998, 2002). The contexts traditionally thought to involve nepotistic conflict of interest include queen duels, worker policing, queen rearing, food sharing, and reproduction in queenless nests. A newer body of work focuses on genomic imprinting. We start with worker policing, as this is often presented as the strongest body of work, and conclude with the newest work on imprinting.

WORKER POLICING

Due to multiple matings by the queen, a honey bee colony is composed of 12 patrilines on average, a patriline being a group that shares both parents (Tarpy and Page, 2001). In this context, a worker is more related to her brothers (the queen's eggs, $r = 0.25$) than she is to her nephews (worker-laid eggs), most of whom are half nephews (mean $r \approx 0.125$ with extreme polyandry). It is therefore predicted that queens should lay male eggs and eggs laid by workers should

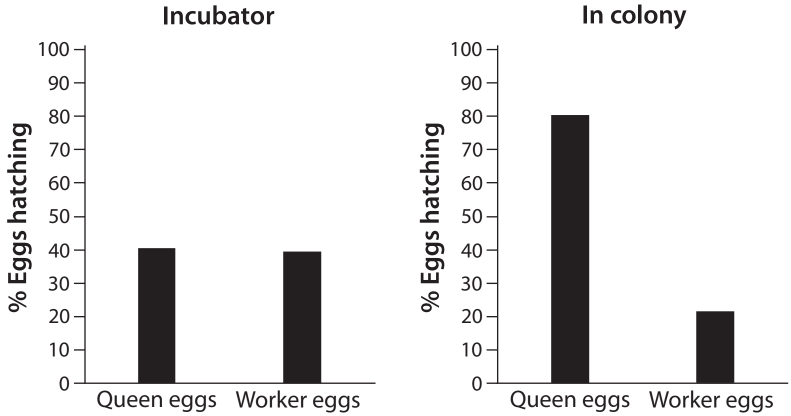


FIGURE 9.1. Hatching rate of queen- and worker-laid eggs in either an incubator (*redrawn from Ratnieks and Visscher, 1989*) or a real nest (*redrawn from Pirk et al., 2004b*).

be killed by the other workers. It is the killing of worker-laid eggs that is called policing (Ratnieks, 1988). Ratnieks and Visscher (1989) showed that worker-laid eggs are quickly removed from the nest by the workers. They also presented data suggesting that worker- and queen-laid eggs are equally fertile.

The initial policing study received much favorable attention, but it has not withstood the test of time. First, Ratnieks and Visscher (1989) tested worker- and queen-laid egg viability in an incubator, an artificial situation in which the hatching rate is low for both queen and worker eggs. When Pirk et al. (2004b) repeated this test in a real nest, they found that worker-laid eggs have lower viability (figure 9.1). More importantly, the kin selection argument for worker policing does not simply predict policing. It predicts context-specific policing, because when the queen is singly mated the workers are more related to their sisters' sons ($r = 0.375$) than they are to the queen's sons ($r = 0.25$) and so should not police. When researchers searched for worker policing in other species in which the queen mates once, they found that many of them police (Kikuta and Tsuji, 1999, Foster et al., 2002, Foster and Ratnieks, 2001, Saigo and Tsuchida, 2004). Even a clonal species, for which there is little to no possibility of a relatedness asymmetry, was found to police (Hartmann et al., 2003). For asexual reproduction in honey bees, after a few studies showing mixed results, it was ultimately shown that Cape honey bees also police, although from a relatedness perspective they are not predicted to do so (Greeff, 1996, Pirk et al., 2002, 2003).

Table 9.1. Prediction table for testing competing hypotheses for worker policing

Relatedness context	Kin selection	Colony-level selection	Experimental results
Singly mated	No	Yes	Yes
Multiply mated	Yes	Yes	Yes
Clonal	No	Yes	Yes

Note: The relatedness contexts in which policing is either predicted (yes) or not predicted (no) are given for both the kin selection and the colony-level selection (superorganism) hypotheses, along with the overall experimental results across species.

How do we make sense of the data on worker policing in honey bees and across the social insects? In short, in situations like this, which occur regularly in the study of kin selection, we need a prediction table, like the one shown in table 9.1. This is because there are multiple hypotheses for what might cause the focal trait and the alternative hypotheses have overlapping predictions. Colony efficiency (related to the superorganism concept) predicts that workers police either because it is more efficient for queens to lay eggs or because queen eggs are better in some way (more likely either to hatch or to produce higher-quality offspring). The superorganism concept thus predicts worker policing independent of relatedness, while kin selection theory predicts context-specific worker policing. Hence, to prove the kin selection hypothesis requires proof that policing occurs when it is predicted to, and that it does not occur when it is not predicted. If it occurs in both contexts, then the mechanism that explains it in the situation in which kin selection does not predict it can also explain it in the situation in which kin selection predicts the behavior. In short, there is currently not clear evidence for worker policing due to relatedness asymmetries, but better support for the colony-level selection (superorganismal) hypothesis (Gadagkar, 2004, Olejarz et al., 2016).

QUEEN DUELS

Kin selection predicts that workers might bias queen duels in favor of their full rather than half sisters. Gilley (2003) tested this hypothesis in a study involving the genotyping of thousands of bees during queen duels. No evidence was found suggesting that the many (often aggressive) interactions between workers and virgin queens are directed toward half sibs. Long et al. (2017) later repeated this study, while also looking for queen quality effects. They found strong effects regarding queen quality and fighting ability, but no evidence of workers favoring more closely related queens.

QUEEN REARING

The hypothesis that workers should bias the production of new queens to those they are more related to (full over half sisters) has received much attention (Breed et al., 1994). Early studies showed conflicting results, with some studies showing weak preferences for full sibs in queen rearing but others no preference (Getz and Smith, 1983, 1986, Breed et al., 1984, Page and Erickson, 1984, 1986, Visscher, 1986, Carlin and Frumhoff, 1990). Many of these studies used the cordovan mutant as a neutral marker for genetic background. It was later shown that the cordovan mutation, and color in general, is not a neutral marker in honey bees, calling into question much of this early work (Frumhoff, 1991, Vanderblom and Verkade, 1991). Further, early studies used experimental colonies with only two or three patrilines, a context that is unnatural given the mating frequency of honey bees. Studies in which the same patterns were sought in colonies with wild-type mating levels did not support nepotistic queen rearing (Hogendoorn and Velthuis, 1988, Chaline et al., 2003). Recent work by Sagili et al. (2018) has confirmed that larvae for queen rearing are chosen based on quality and without reference to relatedness. Even completely unrelated larvae are preferred to related larvae when they are of higher quality, and when quality is controlled for there is no preference associated with relatedness.

SWARMING

During swarming, the old queen leaves and a new queen takes over the old colony. Thus, a worker could find herself in a colony headed by a half or full sister after swarming. Depending on who inherits the nest it might be best to stay or leave. This assumes, of course, no colony-level costs to nepotistic choices (the bugbear in most kin selection predictions). An early study using the cordovan marker and a nest composed of two patrilines found unequal representation of patrilines in the swarm (Getz et al., 1982). A more recent study using naturally mated colonies and swarms found no evidence of nepotism in this context (Rangel et al., 2009). Patrilines were not more likely to stay in the colony if the new queen was a supersister.

ARE QUEENLESS COLONIES FULL OF SELFISH WORKERS?

A perspective stressing conflict in queenless (QL) nests has dominated studies of this topic (Page and Robinson, 1994, Miller and Ratnieks, 2001, Makert et al., 2006). The idea has been that once the queen is lost, it is every worker for herself as cooperation breaks down and everyone tries to maximize their own fitness. We take a different approach. We try to understand what colony-level selection might produce in the hypothetical scenario in which conflict is absent. This can then serve as a null hypothesis for exploring the degree of conflict in this context.

We start with some relevant background information. In a queenright (QR) colony, the division of labor (DOL) system is an adaptation for maximizing work output (Jeanne, 1999, Johnson, 2010a). DOL leads to a short worker life span of about five to six weeks in spring and summer. In contrast, in the winter, when forage is not available, there is no need to maximize productivity. Rather, the goal is to maximize worker longevity since workers must survive the long winter. The resulting caste state, called the winter bee, is a generalist bee that is long lived (Amdam and Omholt, 2002, Mattila and Otis, 2006, Johnson, 2010a).

With this background, what might we expect in a QL nest if the bees are attempting to raise the maximum number of drones (cooperating rather than engaging in a chaotic free-for-all)? First, the colony is not producing new workers, so when the current population dies, the colony dies. Hence, the normal DOL system, which maximizes rate of work output at the expense of longevity, might be maladaptive, and a strategy based on maximizing longevity might be best. Hence, one might predict that bees would be generalists with low activity levels as one sees in winter bees. Second, given that the workforce is slowly dying, it makes sense to lay the maximum number of eggs that can be reared, given the size of the colony, as quickly as possible. Such colony-level traits are typically caused by feedback loops based on pheromone secretion and reception, which is what occurs when the drone larvae inhibit workers from laying too many eggs (Velthuis et al., 1965, Velthuis, 1970a, Jay and Nelson, 1973). With respect to who should lay the eggs, it is clear that it takes many laying workers to lay the necessary eggs as worker ovaries are small relative to those of the queen. Finally, those bees that do not lay eggs should continue to work on tasks necessary for brood rearing only (not the many tasks relating to nest maintenance or growth). This is what the majority of the colony in fact does (Vanderblom and Verkade, 1991, Naeger et al., 2013). Hopefully, this discussion shows that the workers in a QL honey bee colony continue to cooperate.

We turn now to the evidence for conflict. The main idea is that conflict occurs over who gets to lay the eggs. The primary evidence is that some patrilines lay more eggs than others (Robinson et al., 1990, Page and Robinson, 1994). But natural biological variation in the relevant physiological traits would have to produce this result, given that the patrilines vary in their sensitivity to the relevant pheromones and therefore in the rate at which their ovaries will be activated. To show that the unequal ratios of eggs from different patrilines is the result of an adaptation associated with competition, and not just raw variation, more must be shown. It could easily be the case, for example, that patrilines do not show consistent biases across colonies. That is, the highest egg-laying patriline in colony A could be a low-laying patriline in colony B due to the different mix of patrilines in that nest. This would be analogous to a conventional trait that has a genetic basis that is strongly epistatic and not heritable.

This brings us back to the more commonsense notion of looking for deviations from what appears to be highly cooperative behavior. In other words, what would be convincing evidence for nepotism-based conflict in QL nests? There are many possibilities. Observing that the appropriate number of eggs are not laid, or raised, for example, due to selfish interactions between the workers would be convincing. The best evidence for this is simply that there is aggressive behavior in QL colonies, with workers with activated ovaries being the recipients (Sakagami, 1954, Vanderblom and Verkade, 1991, Visscher and Dukas, 1995). This does not seem to interfere with egg laying, however (Vanderblom and Verkade, 1991). Second, and more convincing, some QL colonies fail to produce drones (Page and Erickson, 1988, Page and Robinson, 1994). This is interpreted as being due to conflicts not being resolved in the nest, and this is possible, but it is not the only possibility. QL colonies could also fail due to the state of the colony at the time of queen loss and due to the state of the environment.

In sum, a conflict explanation for some behavior in QL colonies is possible, but it has not yet been shown. All in all, however, if significant nepotistic conflict occurs in honey bee colonies, it is likely in this context, so it would not be surprising if this is shown in future experimental studies.

IMPRINTING

Haig proposed that parent-of-origin bias in gene expression could be due to nepotistic conflict of interest (Haig, 1992, 2000). A classic example is that genes in a developing organism derived from the father (patrigenes) are not related to the mother, in contrast to matrigenes. Thus, patrigenes should favor increased investment to the offspring from the mother relative to matrigenes. Some evidence has been collected in mammals in support of this idea (Moore and Haig, 1991). Queller and Strassmann (2002) pointed out that social insects might be the ideal situation in which to test ideas involving matrigene-patrigene conflict of interest because in a colony such conflict can occur in many contexts. They generated a long list of predictions, sometimes called the kinship theory of genomic imprinting (KTGI), which has triggered much interest (Queller, 2003).

As important background, parent-of-origin effects on ovary size associated with crosses between subspecies have been shown (Linksvayer et al., 2009b, Oldroyd et al., 2014). A similar effect in the context of aggression has also been shown (DeGrandi-Hoffman et al., 1998). Work testing KTGI specifically has looked for parent-of-origin marking of genes and bias in patrigene or matrigene expression. The clearest result perhaps is that methylation in bees does not seem to be involved in marking matrigenes or patrigenes (Kocher et al., 2015, Remnant et al., 2016, Smith et al., 2020). With respect to parent-of-origin gene expression, this has now been found in a few studies. Kocher et al. (2015), in crosses of Africanized and European bees, found matrigene-biased expression across multiple tissues. This is somewhat contrary to KTGI, as it tends to predict patrigene bias in most contexts in honey bees. Galbraith et al. (2016) later looked for patrigene-biased expression in queenless nests. Crosses were again made between European and Africanized bees in both directions, and biased expression due to parent of origin, independent of lineage of origin, was sought. Hybrids with African fathers were more likely to activate their ovaries, and significant patrigene bias in expression was found in reproductive workers. A later, more experimentally detailed study with the same design crossed *A. scutellata* and *A. capensis* queens and drones but looked for patrigene-biased expression in embryos. Of the 21 patrigenes that were found, 6 had gene ontology terms associated with reproduction (Smith et al., 2020).

The reviewed studies are consistent with KTGI but do not demonstrate it (Oldroyd and Yagound, 2021). The reasons were largely outlined in the previous section on worker policing. There are many competing hypotheses that make overlapping predictions (Patten et al., 2014). We do not have space to do justice to them all, but at least two other hypotheses are active areas of research. These are the sexual antagonism theory and the maternal offspring coadaptation theory (Day and Bonduriansky, 2004, Wolf and Hager, 2006). Both of these alternative hypotheses posit that imprinting serves to identify the most fit allele and ensure its preferential usage. For the antagonism hypothesis, the assumption is that there is sex-specific selection making matrigenes more adaptive in females and patrigenes more adaptive in males. For the coadaptation hypothesis, the idea is that epistatic interactions extend beyond the individual to include the mother such that either matrigene- or patrigene-biased expression could be favored, depending on the genetic architecture for the trait. In general, the data thus far in social insects largely have not supported the antagonism hypothesis, but the data seem broadly compatible with the kinship and/or coadaptation hypotheses.

Beyond the alternative hypotheses, there are other reasons to be cautious with respect to the data supporting KTGI. Chiefly, as for worker policing, KTGI makes a series of predictions that cannot be demonstrated in a single species because they are context dependent. Depending on mating frequency (or number of queens), we might expect matrigenes or patrigenes to be selfish. This can only be tested in a series of studies, preferably in a taxonomic group that varies in queen number or mating frequency. Finally, crosses between subspecies may confound testing of these ideas. Hybrids between locally adapted populations can have unpredictable biases in gene expression for a host of poorly understood reasons (Turelli and Moyle, 2007). Given that KTGI predictions should be testable within populations, future work should consider discontinuing the use of subspecies crosses.

In sum, KTGI is an interesting set of ideas and there is some evidence consistent with it. Specifically, parent-of-origin bias in gene expression occurs in the honey bee, and it may be biased toward patrigenes. The key predictions, however, that males and females mark their genes (or direct silencing of genes from the other parent in some manner) have not been shown, and neither has it been shown that the small numbers of identified patrigenes have the expected effects. Until more data come in, it is too early to tell what will become of the KTGI idea in social insects, but it is sure to continue to attract attention.

Conclusions

To quote Breed et al. (1994) in a review of this topic, “Few areas in sociobiology have received as much experimental attention and have yielded as little in the way of supportable conclusions as the question of subfamily nepotism in honey bees.” It may be time to accept the weight of the evidence and entertain the idea that highly derived societies, like the honey bee, are strongly cooperative with only minimal realized conflict constrained to a few domains (probably QL nests and perhaps imprinting). This is not to say that conflict is absent, but rather that the data suggest, and theory affirms, that a superorganism can achieve a state in which the overwhelming majority of their potential conflicts have been resolved (Korb and Heinze, 2004). To quote the classic paper of Queller and Strassmann (1998), "Social insect colonies may fail to meet the standard of perfect harmony, but some might still be as harmonious as organisms." No one should view the genome of an organism as a place with equal levels of conflict and cooperation. Rather, the degree of cooperation is massive, and conflict is constrained to a few clear domains. Superorganisms, like the honeybee colony, may not be as biased towards cooperation as the most advanced metazoan organisms (when viewed as a society of cells), but they may nevertheless have quite small levels of realized conflict.

Population Genetics

Sex Alleles

A sizable body of work in population genetics, using honey bees, focuses on the sex determination system (Cook and Crozier, 1995, Crozier, 1977). As we explained in chapter 3, honey bees are haplodiploid and sex is controlled by variation at one locus. The trigger gene is called the complementary sex determiner (*csd*). Individuals heterozygous for this gene are female, while those hemizygous (haploid) are male. Homozygotes are also male but are infertile and killed by the workers. Several basic topics in population genetics can be studied in this system. First, there should be positive selection for new alleles at this locus (Hasselmann and Beye, 2004). Second, heterozygotes have an advantage over homozygotes and common alleles are more likely to occur in homozygotes, meaning that rare alleles have an advantage due to frequency-dependent selection. These forces together generate a higher level of polymorphism at the site than would be expected due to the forces of mutation and drift alone. This phenomenon, in general, is called balancing selection.

Early work by theorists predicted the number of sex alleles that would be present at equilibrium in idealized populations and those of finite size, along with their frequencies (Yokoyama and Nei, 1979). It was also shown that alleles in this context should rarely be lost to drift and can, in principle, persist for exceptionally long periods of time (longer than the age of most species).

Early experimental work (mainly in Brazil) found numbers of alleles at the *csd* (about 20) comparable to what was predicted (Adams et al., 1977a, 1977b). Later work confirmed the population-specific allele numbers found in earlier studies in a population of wild bees in Kenya, while showing that as a species *Apis mellifera* has a very high number of alleles at the *csd* locus (Lechner et al., 2014, Zareba et al., 2017).

Hasselmann and Beye, in conjunction with their work elucidating the network controlling sex determination, also explored the population genetics of *csd*. They began by showing that balancing selection operates within distinct regions of the *csd* gene, and variation within *csd* is 10–13 times the average level of variation across the genome (Hasselmann and Beye, 2004, Beye et al., 2006). They followed this up with a study in which *csd* sequence variants were compared across *A. mellifera*, and two Asian honey bee species, *A. dorsata* and *A. cerana*. They identified exons six and seven as the sites of balancing selection (binding motifs are here) and inferred that this is the site of the PSD, the potential specifying domain (Hasselmann et al., 2008b). They did not find evidence for transspecific (ancient) alleles and found significantly less variation in *csd* than loci undergoing balancing selection with similar function in other systems, such as the S locus in plants and the MHC complex in mammals. They argued that a modest rate of new allele formation coupled with a small N_e (causing extensive drift) explains the rapid turnover found in *csd* alleles. A final study identified 13 functionally distinct *csd* alleles by extensive crossing of bees with known *csd* genotypes (Beye et al., 2013). They showed that five to six amino acid differences in the PSD are necessary to differentiate alleles and, based on the location of these mutations, likely affect protein binding. Alleles with fewer mutations, and incomplete penetrance, were also found, suggesting an evolutionary route to *csd* allele differentiation (with adaptive intermediates) via single mutations.

Local Adaptation

BROAD STUDIES ON LOCAL ADAPTATION

Zayed and Whitfield (2008) measured F_{ST} for single nucleotide polymorphisms (SNPs) in coding versus noncoding regions across the major lineages of honey bees. This was to test for a general pattern of positive selection associated with the movement of bees out of Africa and into the Middle East and Europe. They found higher F_{ST} values for SNPs in coding regions, suggesting large-scale positive selection. They also showed that introgression of European-derived alleles into the Africanized bee genome has been higher in coding versus noncoding regions, suggesting that rapid evolution of these bees to their introduced range may have involved the adaptive use of genetic variation from both ancestral gene pools.

Wallberg et al. (2014) conducted a study with similar goals, but with increased sampling. They showed that all honey bee populations have high genetic variability and found no evidence for the sort of bottlenecks one would associate with domestication. They put the split between the major honey bee lineages at about 300,000 years ago and found evidence for fluctuations in effective population size associated with glaciation events. Finally, highly differentiated SNPs across populations were associated with several pathways and traits that support previous work on eusocial evolution. Genes with worker-biased expression were associated with highly differentiated SNPs, suggesting rapid evolution in the worker caste (Johnson and Tsutsui, 2011, Harpur et al., 2014, Mikheyev and Linksvayer, 2015). Insulin signaling, vitellogenin (Vg), and immunity pathways were also sites of positive selection (Amdam et al., 2005a, Ament et al., 2008, Harpur et al., 2019).

LOCAL ADAPTATION IN AFRICA

Some recent studies have greatly expanded our understanding of a long known, and fascinating, pattern of local adaptation. This relates to the evolution of *A. m. monticola*, the bee found on East African mountain tops. This bee is larger, darker, less aggressive, less likely to abscond, and able to forage at lower temperatures than the savanna bees (*A. m. scutellata*) found at lower elevations (Meixner et al., 1989, Hepburn and Radloff, 1998, Gruber et al., 2013).

Wallberg et al. (2017) sequenced 39 genomes of bees from four populations (two of *A. m. monticola* and two of neighboring *A. m. scutellata*). They found next to no differentiation across most of the genome for the two groups, suggesting strong gene flow. However, they found two large regions of nearly complete differentiation. Later work confirmed that these are large inversions (0.59 MB and 1.73 MB in size). A follow-up study also found a third (3.4 MB) inversion (Christmas et al., 2019b). Inversions have been shown to play a role in local adaptation in many systems, as they preserve coadapted gene complexes from the effects of recombination (Wang et al., 2013).

Eusocial Evolution

The evolutionary genetics of eusocial evolution has become a major topic over the last several decades (Robinson et al., 2005, Linksvayer and Wade, 2005, Page and Amdam, 2007, Toth and Robinson, 2007, Johnson and Linksvayer, 2010, Wurm et al., 2011, Simola et al., 2013, Linksvayer, 2015, Helantera, 2016, Kapheim, 2016, Dogantzis et al., 2018, Linksvayer and Johnson, 2019). We cover the big picture here. Many relevant experimental data are provided throughout the book relative to these ideas, and it is up to the reader to keep these ideas in mind when later considering that work. We conclude by discussing two evolutionary genetic case histories that are emblematic of the ideas reviewed.

Ladders or Bushes of Social Evolution?

A popular approach to the study of eusocial evolution is to suppose a ladder of social complexity (Rehan and Toth, 2015). The goal in such work is to identify the basis of social behavior in the simplest societies and then follow changes in the same genes or pathways across the transitions to more complex forms of sociality. Overlap between genes involved in honey bee social behavior with those associated with sociality in bumble bees, or other simpler societies, is thus taken as support for the ladder hypothesis (Grozinger and Robinson, 2002, 2007, Grozinger et al., 2007, Toth et al., 2007, 2010).

Recently, Linksvayer and Johnson (2019) reconsidered this notion of a ladder of social complexity. Of chief concern is that predictable "ladders of progress" along any front are traditionally held up as cautionary tales in the study of evolution (Gould, 1990, 2002). Evolution rather tends to produce phylogenies resembling bushes in which many possible pathways through phenotypic space are explored (Baum et al., 2005, Brady et al., 2006, Cornwell and Nakagawa, 2017). A bee species with a complex society might have a precursor with an organization like *Bombus*, for example, or all species with the ancestral state might be extinct. It could further be unlikely to evolve one form of derived eusociality if a species is on a trajectory more often associated with some other form of eusociality. This could explain why some lineages evolve swarming behavior and others never do. The reason some clades have nursing behavior associated with glandular secretions, as opposed to bulk providing, could likewise stem from their divergent starting points. The possibilities are endless, and the point here is that evolutionary trajectories are complex and notions of simple progressive change seem unlikely. The take-home message, of course, is that a genus like *Bombus* might not be a good proxy for the ancestor of a genus like *Apis*.

Ground Plans

The ground plan idea supposes that there are core genetic modules that control large components of behavior and physiology and, when novel phenotypes evolve, it is through tinkering with these modules (West-Eberhard, 1987, 1996, 2002). Genetic modules are proposed to change in their connections to other modules, or in their time or place of expression (Hunt, 2007). In honey bees, for example, it is argued that Vg, and ovarian activation in general, are at the center of a conserved ancestral network controlling both reproduction and many aspects of sociality (Amdam et al., 2003a, 2004b, 2006a, Nelson et al., 2007, Bloch and Grozinger, 2011, Graham et al., 2011, Kapheim and Johnson, 2017, Oldroyd and Beekman, 2008, Rueppell et al., 2008, Tsuruda et al., 2008).

Although the notion of a ground plan is presented in simple terms, it is often unclear what constitutes a ground plan in concrete terms. If the genetic basis of a precursor trait is identified in a solitary species, for example, and half of those genes are also involved in social behavior, is that weak or strong support for the idea of a cohesive genetic module that selection tinkers with but does not reengineer? What of the 50% of genes not conserved (Warners et al., 2019)? Further, what if the percentage of overlap is much less? What if the overlap in genetic basis is only for some classes of genes but not others? All of these issues are at play in interpreting the data on ground plans. Further, the strongest support for the ground plan idea comes from studies of single genes, such as Vg, or phenotypic states, such as ovariole number. However, the ground plan idea is fundamentally one that stresses conservation of whole pathways (modules or networks), not individual genetic actors or phenotypic states (Berens et al., 2015).

The goal of this section is not to dismiss the ground plan idea. Something like this is probably going on to some extent. Rather, the goal is to point out that greater rigor is required to test it. Most importantly, a focal gene cannot be used as a proxy for the whole pathway if the goal is to show pathway conservation.

Differentially expressed transcripts

	Apis mellifera (honey bee)	***Polistes metricus*** (paper wasp)
Polistes metricus (paper wasp)	110 (88) of 14,947	
Solenopsis invicta (fire ant)	302 (436) of 2,493	12 (19) of 4,706

FIGURE 9.2. Differentially expressed genes in common between honey bees, paper wasps, and fire ants based on whole-brain extractions (*from Berens et al., 2015*).

Toolkits

The toolkit idea stresses that some genes and pathways are reliably associated with particular traits and that when evolutionary change occurs it is often associated with changes to members of the toolkit (Robinson, 1999, Robinson et al., 2005, Toth and Robinson, 2007). The ground plan idea is thus an extreme form of toolkit in that conservation of the whole network is proposed. Most research in molecular biology is supportive of the notion of a genetic toolkit, and it is no different for social behavior. We focus on some work that addresses the broadest issues. Chapters 4 and 12 review a great deal of work in this area.

Many studies have compared socially relevant genes (differentially expressed genes between castes or in response to pheromones) between bumble bees, *Polistes* wasps, and honey bees or ants, and found moderate levels of overlap (Grozinger et al., 2003, 2007, Toth et al., 2007, 2010, Warners et al., 2019). Berens et al. (2015) made a somewhat more detailed comparison between two advanced eusocial species (fire ants and honey bees) and two primitively eusocial *Polistes* species. They looked for overlap in differentially expressed transcripts in the larval brain between workers and queens in each of these systems. Surprisingly, they found few conserved genes (figure 9.2). Conservation was present, however, in the relevant pathways involved in queen-worker differentiation. These included several core metabolic pathways and genes associated with transcriptional regulation. They concluded that, although particular genes may not repeatedly evolve changes in function to facilitate social evolution across clades, some genes within the relevant pathways do. Dogantzis et al. (2018) found something similar in that *Bombus* and *Polistes* were found to share some general evolutionary patterns of change in regulatory genes, relative to a more advanced social species, the honey bee.

Genomic Reorganization

The evolution of eusociality is associated with a significant expansion, at the colony level, in behavioral and physiological complexity (Michener, 1969, Wilson and Hölldobler, 1988, Hölldobler and Wilson, 1990, Johnson and Linksvayer, 2010). As we discuss in chapter 15 on foraging, for example, honey bees likely retain much of the behavior associated with foraging in solitary bees, as it is made use of by scouts. In addition, however, honeybees have evolved a new level of collective regulation of foraging, involving multiple mechanical signals and pheromones. Friedman et al. (2020) further pointed out that the physiological regulation of development and reproduction also involves expansion of function, in that the internal endocrine pathways controlling such processes in solitary insects are intact in social insects, but colony-level social physiological feedbacks are superimposed onto them to facilitate group-level coordination of action (Seeley, 1995). The nature by which brood and queen pheromones shape development and behavior by acting as priming and releasing pheromones are clear examples of such distributed social physiology.

The novelty of superorganismal organization suggested to Johnson and Linksvayer (2010) that eusocial evolution may be associated with a massive rewiring of the genome—the idea here being that the toolkit notion is largely correct, in that many of the same genetic actors involved in social behavior were involved in ancestral traits, but that many actors have evolved new functions and network interactions to facilitate the collective control of behavior, physiology, and development. This idea has started to gain support, as Simola et al. (2013) found that regulatory genes are rapidly evolving across the ants. Harpur et al. (2014) likewise found that genes with worker bias expressed are enriched for positive selection in cis-regulatory regions. This pattern did not hold for bumble bees, where queen-biased genes are more likely to be positively selected (Harpur et al., 2017). Finally, Kapheim et al. (2015a) found that regulatory regions are more complex in eusocial taxa than in solitary taxa and that they are the most complex in the most advanced social groups. These results are preliminary, in that such an idea must be tested in larger, phylogenetically controlled datasets (Linksvayer and Johnson, 2019), but future work will undoubtedly continue to test this intriguing notion that the major evolutionary transition leading to eusociality is associated with a major genome reorganization, just as were all the other major evolutionary transitions (Smith and Szathmary, 1997).

Taxonomically Restricted Genes

Taxonomically restricted genes (TRGs) are genes limited to a particular clade (Khalturin et al., 2009). In the case of orphans, these are genes found only in one species, but restriction can be at any phylogenetic level. TRGs have been found to comprise a significant fraction of all genomes (10%–20% of genes) and have been shown to be associated with the evolution of novelty across the tree of life (Wilson et al., 2005, Voolstra et al., 2011, Ranz and Parsch, 2012). The gall-inducing insects, for example, have a massive expansion of novel genes thought to underlie their ability to control plant physiology (Zhao et al., 2015). For eusocial evolution, the role of TRGs is the opposite of the toolkit idea. In this case, it is thought that when novelty is selected for, new genes emerge to play the novel roles (Wilson et al., 2005, Khalturin et al., 2009, Johnson and Tsutsui, 2011, Voolstra et al., 2011, Sumner, 2014, Jasper et al., 2015). These new genes could be old genes that change so radically in function that they no longer have functional similarity to their homologs, or they could be genes that arise de novo (Begun et al., 2007).

In the case of social insects, Johnson and Tsutsui (2011) predicted, and found support for the idea, that since workers are the caste (at least in the most derived insect societies) that exhibits the most novel behavior and physiology, they should express more novel genes. Other studies in ants, bees, and wasps subsequently found support for a major role for TRGs in eusocial evolution (Ferreira et al., 2013, Simola et al., 2013, Mikheyev and Linksvayer, 2015). Harpur et al. (2014), for example, found that Apoidea and *Apis*-specific genes are rapidly evolving relative to genes found across the Insecta. Jasper et al. (2015) then proposed that TRGs should play their largest roles in tissues with novel functions (mainly glands serving social functions) and they should primarily be effector genes at the distal branches of gene networks. This complements the evo-devo approach, as that idea stresses little coding sequence change in the hubs of networks due to pleiotropy but is agnostic as to what goes on at the ends of networks where pleiotropy is often not an issue. Transcriptomic data for 10 tissues were generated and TRGs were found to be central to tissues with social functions, with the exception of the brain (Jasper et al., 2015). The hypopharyngeal (HPG) and sting glands were exceptional, as most of the highest-expressed genes in these tissues are TRGs. The HPG serves social functions, while the venom gland serves a lineage-specific function, both of which involve phenotypic novelty (Johnson, 2018).

Case Histories

We conclude with discussion of two gene families that can serve as case histories for the ideas we have been discussing. The first relates to the yolk protein, vitellogenin (Vg), which is a classic case of evolution in the toolkit paradigm, while the second relates to the major royal jelly proteins (MRJPs), which have a complex history associated with novelty and major changes in gene function. The goal is to explore why all the major ideas discussed here are necessary to understand eusocial evolution at the genetic level.

VITELLOGENIN

Vg is a lipid transport protein that is the precursor for vitellin (the main yolk protein) across much of the tree of life (Hagedorn and Kunkel, 1979, Wahli, 1988). In insects, it is produced in the fat body, secreted into the hemolymph, and then taken up where needed (mainly the ovaries) via receptor-mediated endocytosis (Sappington and Raikhel, 1998, Tufail and Takeda, 2009). It is a protein with many functions both ancestrally and in response to selection in the social insects. In its ancestral role, posttranslational modifications allow Vg to carry lipids, carbohydrates, and other nutrients (Tufail et al., 2014). This is associated with transporting these substances to the egg. However, given that Vg has the ability to bind all of these compounds, and given Vg's high titers in the blood, it is not surprising that numerous other functions have evolved. In fish, for example, Vg can bind to cell walls to help shield against oxidative stress (Sun and Zhang, 2015). Vg has also been shown to bind to harmful microbes as part of the immune response (Li et al., 2009). It seems to have evolved a pattern recognition molecule function in this context (Li et al., 2008, Liu et al., 2009, Zhang et al., 2011, Salmela et al., 2015). The role that Vg plays in honey bee behavior and physiology is a classic example of the toolkit paradigm and serves as our case history for evolution of this type.

The primary role of Vg is in the production of egg yolk protein; hence, its major burst of expression coincides with the activation of reproductive physi-

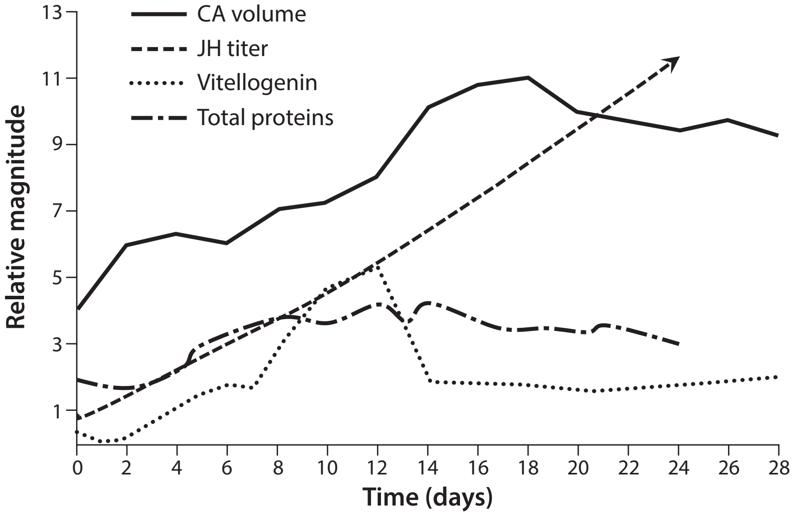


FIGURE 9.3. Vg and JH levels, and corporal allata volume, in aging bees (*from Rutz et al., 1976*).

ology (Hagedorn and Kunkel, 1979). In most insects, this surge of Vg expression is regulated by either JH, ecdysteroid, or both (Tufail et al., 2014). In honey bees, this is still the case in queens; however, Vg has evolved a major novel function in workers, who are typically sterile (Tanaka and Hartfelder, 2004). Here Vg expression peaks in the nursing phase (in young bees), as shown in figure 9.3 (Rutz and Luscher, 1974, Rutz et al., 1977, Fluri et al., 1982), the opposite of the ancestral pattern. Further, nurse bees have high Vg levels, which fall off at the transition to the middle-age caste (Rutz et al., 1976). Later, during the middle-age caste, there is a surge of JH, which sometimes precedes and sometimes lags the transition to foraging (figure 9.4; Huang et al., 1994). Knockdown of Vg at emergence causes precocious foraging, although it is not clear if this is due to skipping only the nursing caste or both the nursing and middle-age castes (Amdam et al., 2003b, Guidugli et al., 2005). With respect to the function of Vg in nurses, it serves as a storage protein underlying the production of brood food (Amdam et al., 2003a). There is also some evidence that Vg is associated with other social traits, such as foraging (Nelson et al., 2007, Ihle et al., 2009). Hence, a novel reproductive trait, secretion of proteinaceous brood food, evolved from a related ancestral function, the stocking of the egg with yolk. That the major endocrine players have also evolved novel patterns to realize this change is a clear demonstration of the power of the toolkit concept.

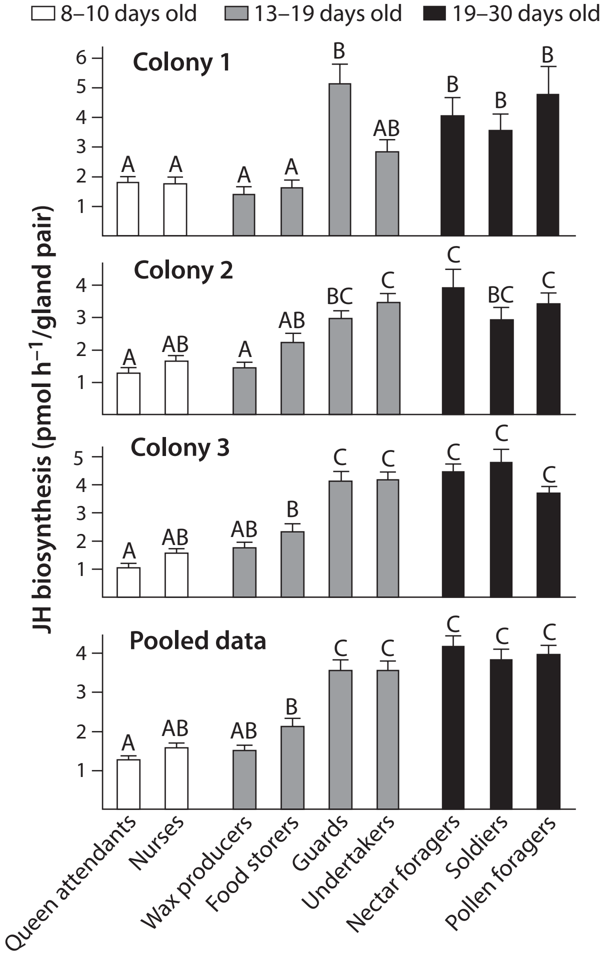


FIGURE 9.4. JH synthesis in bees in different castes and or engaged in different activities (*from Huang et al., 1994*).

Vg has also been shown to play the same roles in bees in immunity and oxidative stress defense that it plays in other organisms (Havukainen et al., 2013, Morandin et al., 2014, Munch et al., 2015, Salmela et al., 2015, 2016). Seehuus et al. (2006), for example, found that Vg is associated with protection against oxidative stress (figure 9.5). Exactly how important Vg is to such traits is unclear, however. Drones older than 14 days, for example, have no circulating Vg, but they have completely functional immune systems (Gaetschenberger et al., 2012). It would be interesting to check if they can protect themselves against

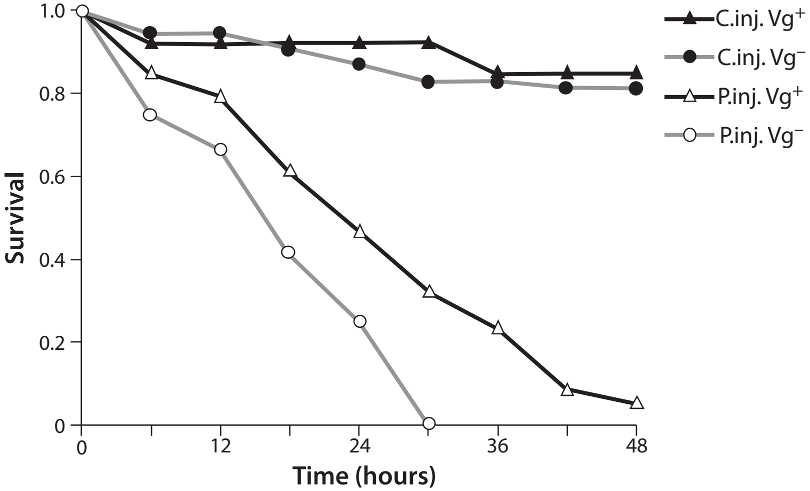


FIGURE 9.5. Survival of Vg knockdown bees in conjunction with paraquat injection to induce oxidative stress (*from Seehuus et al., 2006*).

oxidative stress as well as workers. Vg has also been proposed to have an antiaging function in a caste-specific manner, but this is unresolved. Some studies suggest yes (Amdam et al., 2004c, Munch and Amdam, 2010, Ihle et al., 2015a), while others suggest no (Aurori et al., 2014, Cardoso et al., 2018). We review related work on the role of Vg in these contexts in chapter 17 when we discuss the "throwaway caste" idea (Amdam et al., 2005a, Seehuus et al., 2006). Although it may not be the case that Vg plays fundamental roles in all the processes it has been suggested to, it is likely that it plays some role (probably socially derived) in at least some of these contexts. Future work may thus confirm more toolkit-like evolutionary patterns associated with social behavior in these contexts. Of course, there is a lot of what can be called genomic reorganization in these examples as well.

Finally, population genetics work exploring changes to Vg sequence provides additional support for the importance of this gene in social evolution. Kent et al. (2011), for example, in a small-scale study found support for Vg being positively selected and having undergone a recent selective sweep within the genus. These results were further supported by the genome-scale study of Harpur et al. (2014), as Vg stands out at the genomic level as a gene that has experienced quite strong positive selection. Further support for the importance of Vg comes from other social insects, where the gene has been shown to have experienced duplications and neofunctionalization (Wurm et al., 2011).

THE MAJOR ROYAL JELLY PROTEINS

The MRJPs are a gene family limited to the Hymenoptera (Albert et al., 1996, 1999a, 2014, Albert and Schmitz, 2002, Albert and Klaudiny, 2004, Drapeau et al., 2006). They are part of a slightly larger gene family that includes the yellow proteins, from which the MRJPs evolved (Ferguson et al., 2011, Buttstedt et al., 2014). All insects have yellow proteins, as do about 45 species of bacteria (Drapeau et al., 2006, Ferguson et al., 2011). Other than that, the yellow proteins are only found in a few scattered clades, including two groups of fungi, amphioxus, and an amoeboflagellate. Conserved genes (which comprise the majority of any genome), to put this pattern into perspective, are found in all metazoans almost without exception (Khalturin et al., 2009). The yellow proteins are thus a taxonomically restricted gene class with a scattered taxonomic distribution. This is quite rare. To account for this pattern, it has been hypothesized that either these genes jumped horizontally from bacteria into the insects and a few other clades early in their evolutionary history or they are an ancient clade that has been lost from most groups across the tree of life (Ferguson et al., 2011).

MRJPs occur in tandem arrays and are quite variable in number (Buttstedt et al., 2014). The honey bee genome, for example, encodes 10 MRJPs (9 functional genes and 1 pseudogene) in an array flanked by yellow proteins (Drapeau et al., 2006). In the ants, comparable numbers of MRJPs are found in some species, while in others the array is present but most of the members have become pseudogenes. *Solenopsis invicta* (the red imported fire ant), at the extreme, has lost all copies. Copy number is not associated with sociality, as solitary Hymenoptera, such as *Nasonia* sp., parasitoid wasps, have many (10) copies (Werren et al., 2010, Bonasio et al., 2010, Suen et al., 2011). Many hymenopterans, such as *Bombus* spp., have only a single copy, and this is thought to be the ancestral state (Kupke et al., 2012, Buttstedt et al., 2014).

With respect to function, the yellow proteins were first identified in *D. melanogaster*, where they are involved in pigmentation and sex-specific mating behavior and development (Drapeau et al., 2003, Ferguson et al., 2011). Given their patterns of expression in *Drosophila*, they likely have other functions as well (Wittkopp et al., 2002, Prud'homme et al., 2006). MRJPs are much like the yellow proteins with respect to function when considered as a family. They are expressed in many tissues and in all life history stages. There are, however, in honey bees sharp, tissue-specific expression patterns at the individual gene level. MRJPs one through three and five are highly expressed in the hypopharyngeal gland and are the classic royal jelly proteins (Kubo et al., 1996, Schmitzova et al., 1998, Winkler et al., 2018). Most MRJPs are expressed in the brain, with several showing tissue-specific localization (Kucharski et al., 1998, Peixoto et al., 2009, Garcia et al., 2009, Hojo et al., 2010). MRJPs one and three are expressed in queens and drones, while one, three, and seven are expressed in larvae and are involved in immunity (Drapeau et al., 2006, Randolt et al., 2008). Finally, MRJPs eight and nine are expressed in the venom gland and are known allergens for humans (Peiren et al., 2008).

Little is known overall of the mechanistic function of yellow proteins or MRJPs. The MRJPs in royal jelly serve a clear nutritional role, as a nitrogen-rich repeat motif is present in those genes and is enriched in bee essential amino acids (Albert et al., 1999b, Schmitzova et al., 1998). MRJPs may also play an antibiotic role in royal jelly (Chan et al., 2009). Finally, MRJP-1 was recently shown to have a structural function, as it polymerizes in royal jelly with apisimin (a novel gene also associated with immunity) to form a sticky, filamentous matrix that holds the queen larvae upside-down in her cell during development (Buttstedt et al., 2018). The function of the other MRJPs is unknown, as is what the genes with known functions in the HPG do in other tissues.

Having reviewed the history of the MRJP family, how might we interpret these data relative to our previous discussion of eusocial evolution? The notion of a toolkit is associated with highly conserved genes with relatively stable functions through evolutionary history. Genes may be duplicated and reused in the toolkit paradigm, but in a tinkering manner, not in a wholesale change of function. This set of ideas does not fit well with the MRJPs. They are not highly conserved, being absent from most animal clades, and they have wildly variable functions even in the groups in which they are present. Rather, these genes seem to be hot spots for the evolution of new functions across the insects. They duplicate readily and neofunctionalize rapidly in many contexts. The nutritional role of the honey bee MRJPs, in particular, seems unique to *Apis* as a genus, as most other bees do not feed proteinaceous secretions to their larvae. Finally, the MRJPs seem to be regulated by the same endocrine mechanisms controlling many socially relevant toolkit genes (Winkler et al., 2018). Given that the MRJPs are produced from Vg in the HPG, it is not surprising that both genes share some regulatory overlap (Amdam et al., 2003a, Seehuus et al., 2007). Thus, these genes—whether novel or radically changed in function—are connected to networks also controlling toolkit genes. This is quite a clear example of how all three mechanisms (toolkits, genomic reorganization (de novo construction of novel gene networks relating to sociality), and TRGs) are manifest, and play complementary roles, in social evolution.

10

Life History, Ecology, and Nesting Biology

Honey Bee Life History

The Annual Cycle

We have touched on the honey bee colony cycle at several points in this book. Here we give a brief synopsis of the major phases of colony activity across the year. We reference the work of Seeley and Visscher (1985) on the life cycle in the northern part of the temperate zone. In this study, weight gain, brood rearing, swarming, and other life history variables were tracked for several years. The take-home messages are as follows. First, most of the food collected by a colony occurs during intense but short nectar flows (figure 10.1). For much of the rest of the year, the colony is surviving on its stores and losing weight. Reproduction occurs seasonally, in late spring, and is associated with the peak of brood rearing and with plentiful forage in the field. The large number of sealed brood cells allows the colony to quickly recover after the loss of most of the workforce due to swarming. Needless to say, the timing of these events varies with latitude. Second, the minimum colony size occurs in midwinter just before brood rearing begins, typically in January or February. Further, brood rearing is the costliest aspect of overwintering as the colony must heat the brood zone to at least 33°C, which is far above the 10°C that adult bees are able to tolerate. Thus, if starvation is going to occur, it typically happens during this phase. Finally, because a honey bee colony begins brood rearing during winter, at a time when other insects are in a chill coma (the insect version of hibernating), they get a major head start relative to other social insects. A honey bee colony reaches peak size far earlier than does a bumble bee colony, for example.

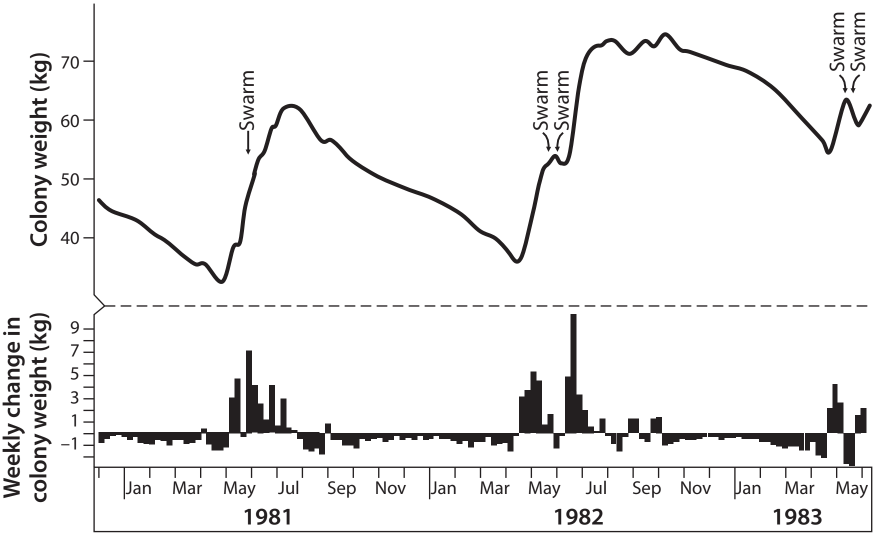


FIGURE 10.1. Weekly changes in total nest weight over the active season (*from Seeley and Visscher, 1985*).

r/K *Selection*

Seeley (1978) pointed out that honey bee colonies do not follow a classic *r*-type life history strategy. The *r*-type strategy is characterized by high rates of reproduction and mortality and fluctuations in population size (MacArthur and Wilson, 1967). This strategy is typical of most social insects. For example, colonies across most clades produce large numbers of queens each year (or periodically) and thus have the potential for massive population growth. What prevents this is quite high mortality for queens and young colonies. Honey bees, in contrast, like swarm founding wasps and army ants, reproduce as a unit of many thousands of individuals. While the first-year mortality of a swarm is high, it is nowhere near what it is for the thousands of new queens (and incipient colonies) produced each year by a typical social insect colony. Further, honey bee populations do not fluctuate much from year to year. In a temperate deciduous forest, for example, the annual survivorship of an established colony was 78% (Seeley, 1978). In general, honey bee life history is characterized by the *K*-type strategy: low mortality, low reproductive rate, long life span at the colony level, high population stability, and consistent year-to-year reproduction. This is probably true for swarm founding social insects in general.

Being cognizant of the honey bee's *K*-type life history strategy is important because it reinforces the notion that not all social insect colonies are organized in the same way. Bumble bees, for example, are far less *K* oriented than are honey bees, and this probably dictates, in part, why their foraging biology is so different. This is even more true for comparisons of honey bees with solitary bees, as a honey bee forager's decisions depend on a host of factors missing from consideration for a solitary bee, and vice versa (Friedman et al., 2020). Members of social species, for example, can be assigned risky tasks reliably leading to death (or be hyperspecialized), while a solitary bee must be a jack-of-all-trades and has no safety net for the care of their young should they die (Queller, 1989).

Topics in Ecology

Population Biology

The density of honey bee colonies varies widely across the native and introduced ranges. In Europe, most studies report population density in the range of 2–3 colonies per km^2 (Moritz et al., 2007, Jaffe et al., 2010). This is true for all the subspecies for which there are data. In Africa, however, the population density is higher, with 12 colonies per km^2 reported in a tropical forest in Uganda and 12–17 per km^2 in a South African savanna (Kajobe and Roubik, 2006, Moritz et al., 2007). Invasive honey bees in North America seem to be at a similar population density to Europe in places with similar climate (Morse et al., 1990, Seeley, 2017), but they can reach much higher levels in some parts of Australia, 75–150 colonies per km^2 (Oldroyd et al., 1994a, 1997). Africanized honey bees were found to be present at about 6 colonies per km^2 in Chiapas, Mexico, but this is quite variable across their range (Ratnieks et al., 1991, 2016b, Winston, 1992).

A study by Oldroyd et al. (1997) is particularly revealing for the population biology of invasive European *Apis mellifera*, as colonies were followed for several years. In addition to the high numbers of feral colonies (75–150 per km^2), it was shown that a swarming rate of just 0.75 swarms per colony per year is sufficient to maintain the population. Considering the actual rate is likely 2–3 swarms per colony annually, the population has a robust capacity for growth. Accordingly, when the authors eradicated bees from a large area, they recorded a recovery rate of 15 colonies per km^2 per year. Thus, a healthy population of honey bee colonies, in a suitable habitat, is capable of rapid growth after periods of decline. The rapid recovery of wild and feral honey bees following the decline due to *Varroa* arrival is thus not surprising.

Wild Populations

It is commonly believed that wild honey bees in Europe and North America were wiped out by Varroa mites and/or were eliminated due to introgression from managed colonies (Kraus and Page, 1995, Jaffe et al., 2010, Potts et al., 2010b). We touched on this question in chapter 1 when we discussed whether the honey bee is domesticated. Surprisingly, this notion is based on just a handful of geographically limited studies, or on studies that only infer low numbers of wild colonies (Kraus and Page, 1995, Loper et al., 2006, Moritz et al., 2007, Jaffe et al., 2010).

Several recent studies have explored the health of wild *A. mellifera* in Europe (Groeneveld et al., 2020, Henriques et al., 2018, Pinto et al., 2014). Browne et al. (2020), for example, found a thriving population of wild colonies in Ireland with little introgression from managed colonies. Managed colonies are typically hybrids of western and eastern European lineages, making them easily distinguishable at the genomic level. A large-scale study explored the genetic integrity across Europe of wild populations, focusing on *A. m. mellifera*, the subspecies thought to be most at risk (Pinto et al., 2014). The researchers found modest introgression between European lineages across Europe with strong variation across countries (figure 10.2). Many countries showed healthy populations of wild colonies of *A. mellifera* (of both eastern and western lineages) with little introgression. Of course, the goal of protecting and preserving wild populations of honey bee colonies is important and should be encouraged (De la Rua et al., 2009), but this is a separate question from whether healthy populations of wild colonies exist in nature. It might be better to stress, going forward, that we must protect these healthy populations from threats, rather than argue that they are already critically damaged.

Interspecific Competition

The western honey bee has been introduced nearly everywhere humans beings live. There has long been interest in the effects of these introductions (and the elevation of natural honey bee populations via beekeeping in the native range) on the abundance of other bee species (Graystock et al., 2016, Mallinger et al., 2017, Geslin et al., 2017). Given that honey bees share a common food

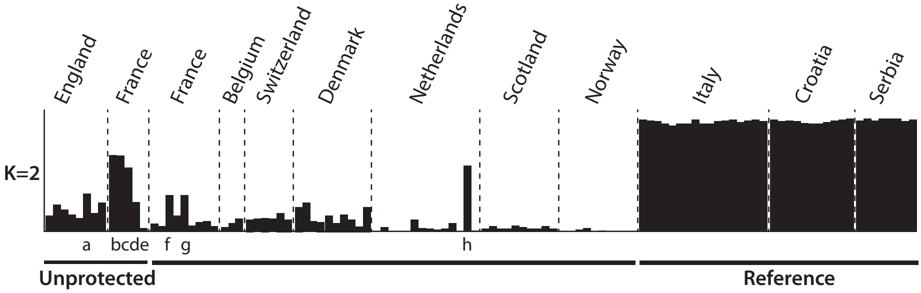


FIGURE 10.2. Unmanaged (wild) honey bees in many European countries show low levels of admixture from other subspecies based on an analysis using STRUCTURE with 1183 SNPs and K=2 partitions. Each bar represents an individual from the population, and black or no shading indicates percentage of ancestry likely from one population or the other (*from Pinto et al., 2014*).

resource with other pollinators, it stands to reason that these introductions must have some effect. Further, for other cavity nesting bees, there is also the concern that honey bees may dominate nesting sites. There has recently been a surge of interest in this topic, with a large number of studies from across the world. Most of these studies confirm the rather commonsense conclusions drawn by earlier work, but they have also raised new concerns, such as the effect of honey bees on the spread of pathogens.

A recent review by Mallinger et al. (2017) comprehensively explored studies of the effects of honey bees on other pollinators. First, with respect to honey bee competition for floral resources or nesting sites, about half of the studies thus far report negative effects of *A. mellifera* on other bees, while the rest report either no or variable effects. Second, there is concern that invasive honey bees may change plant community structure in ways detrimental to native bees. Studies of this topic are split almost into even thirds with respect to those showing positive, negative, or no effects of *A. mellifera* on other species of bees. Finally, most studies exploring the spread of disease by *A. mellifera* to non-*Apis* species have found negative effects (that is, spread from *Apis* to the wild bees). This is typically measured as the presence of pathogens associated with *A. mellifera* in other pollinators or as higher rates of pathogens in wild bees living next to apiaries (Singh et al., 2010, Levitt et al., 2013). In general, most of this work (in all three categories) is limited by the collection of fitness proxies only (visitation rates on flowers, for example) and by the lack of clear data on native bee abundance. We conclude with some discussion of studies that do show such effects.

The clearest results associated with the measurement of fitness relate to *Apis* suppressing *Bombus* populations. Thomson (2004), for example, in coastal California showed that the fitness of a *Bombus* colony is negatively affected by proximity to *A. mellifera*. She also followed the recovery of honey bee populations after their fall due to the arrival of *Varroa* (from 1999 to 2014) and showed that, as honey bee populations recovered, *Bombus* populations declined (Thomson, 2016). Similar effects on *Bombus* have been found in Europe (within the native range of *Apis*), although the effects are dependent on habitat (Goulson and Sparrow, 2009, Elbgami et al., 2014, Herbertsson et al., 2016). Less clear are the effects of managed or introduced *Apis* on solitary bees. In the tropics, Roubik and Wolda (2001) recorded population abundance of the total bee community on Barro Colorado Island in Panama for 17 years. Africanized honey bees (AHBs) arrived in year 7, allowing for a nice, natural experiment. Although clear competition at flowers was observed, there were no effects on native bee populations that could be associated with the arrival of AHBs. In a later study, Roubik and Villanueva-Gutierrez (2009) showed that when AHBs arrived in Mexico they dominated some floral resources, but native bees switched to other plant species and did not suffer a fitness deficit. Studies in other parts of the world have shown negative effects of *Apis* on native solitary bees, however. Paini and Roberts (2005), for example, showed a decline in the fitness of solitary bees (*Hylaeus alcyoneus*) associated with the presence of honey bees in Australia. Lindstrom et al. (2016) showed large-scale suppression of pollinator abundance (in bees and flies) in fields of oil seed rape in northern Europe in association with high density of managed honey bee colonies. In conclusion, it is clear that honey bees compete for common resources with other pollinators and that they negatively affect bumble bees. It is also clear that they sometimes negatively affect solitary bees. It is hoped that future work will clarify the biotic and abiotic contexts associated with the most severe negative interactions.

Intraspecific Competition (Robbing)

Although robbing has attracted little attention from researchers, it is an important behavior. First, rich nectar sources are only intermittently available. The rest of the season is one of dearth, and during these times robbing is commonplace. It is unknown how much food is collected this way, but it could be considerable. Furthermore, *Varroa* and other parasites and pathogens are spread through robbing (Peck and Seeley, 2019). Weakness due to illness may even be the prime cause of a colony being robbed. For these reasons, this subject is deserving of closer study.

NATURAL HISTORY OF ROBBING

When flowers are in bloom, colonies do not typically post guards at the entrance (Butler and Free, 1952, Free, 1954, Moore et al., 1987). The costs and benefits of fighting do not favor conflict at such times. When the dearth comes, however, foraging shuts down and only the scouts stay busy (Seeley, 1983, Beekman et al., 2007). They continually look for new floral resources, but they also search for other colonies within flight range. Here we encounter our first unknown. It is not known if the scouts that look for food and those that monitor other colonies are the same or different bees. Further, it is not known how far the scouts go in search of the nests of conspecifics.

The scouts (or robbers) who find other nests seem to probe the defensive ability of the nest. This is easily observed in apiaries during times of dearth. A robber bee has a characteristic zigzag flight pattern as she approaches a foreign nest (Free, 1954). When the guards see this behavior, they fly out and attack. Many fights thus take place in midair, or in the grass as both bees fall to the ground attempting to sting one another. Although fights often end in death for the loser (Butler and Free, 1952), robbers are sometimes able to avoid the fight. The zigzag flight, which is sure to attract attention, could therefore be a signal to the opposing colony's guards. If a guard flies out immediately in response to the stereotyped flight pattern, then the colony is likely strong and perhaps should not be attacked. If no guard flies out, then the colony may be weak—information that should lead to an attack. This is only speculation, of course (Johnson and Nieh, 2010). The zigzag flight might simply be associated with unfamiliarity of the robber with the new nest (Gary, 1966a). In any event, once inside the nest, a robber steals honey and flies back to her own nest. Presumably, she then uses the waggle dance to trigger an attack, but this has not been directly observed. When an attack begins, it can be massive, with thousands of bees fighting at or near the entrance. When the older bees (the defenders) are killed off, the nest is robbed out. This includes taking all the honey. It is unclear if honey bees also eat the larvae of the colonies they rob. The young bees and the queen are often left alone to starve in the robbed-out nest.

EXPERIMENTAL WORK ON ROBBING

Having discussed the natural history, we turn to the minimal experimental work. In a classic study, Butler and Free (1952) showed that defense is inducible in honey bees. They released 10 foreign bees at a time at a hive entrance and recorded their probability of being mauled. Initially, no bees were mauled, but once the colony became alerted, all bees attempting entry were mauled (Butler and Free, 1952). Interestingly, many bees that are mauled do not fight back and are not killed. This might be an adaptation for preventing too many friendly-fire incidents, because when a full-fledged attack is on, there is chaotic attacking of all bees trying to enter the nest. Bees from the attacked colony do not seem to fight back, while robbers try to kill their attackers.

Optimal Acceptance Thresholds

Early work on "recognition of self" stressed that individuals may calculate some level of dissimilarity to a template in order to determine acceptance or rejection (Lacy and Sherman, 1983, Getz and Smith, 1983). Reeve (1989) expanded on this idea to make some predictions regarding nestmate recognition in social insects. He posited that since the labels used to signal identity are shared between sender and receiver (both nestmates and nonnestmates use the same chemical cues), there is likely some overlap in their distribution between colonies. Hence, it may not be possible to perfectly identify all nestmates without making false acceptances of nonnestmates and false rejections of nestmates. An optimal acceptance threshold must therefore evolve that maximizes fitness with respect to the frequency, and cost, of each type of error. Reeve predicted that in times of plenty the acceptance threshold should be low, allowing many false acceptances, while in times of dearth (and competition) the acceptance threshold should be high, so false rejections should occur. Experiments have supported this idea in honey bees. During periods of dearth, 80% of nestmates and 25% of nonnestmates were accepted by guards, while all bees, including nonnestmates, were accepted when nectar foraging improved (Downs and Ratnieks, 2000). It is unclear what the 20% of rejected nestmates did during the dearth period. It is unlikely colonies permanently reject (kill) 20% of their members each dearth (B. R. Johnson et al., 2011, 2012). Work showing a rapid change in acceptance threshold in response to challenges by nonnestmates also supports this model (Couvillon et al., 2008).

Predator-Prey Dynamics

The honey bee colony's massive stores of honey and larvae (rich in fat and protein) are a nutrient bonanza in wild landscapes, and the bees have evolved many adaptations for defense. The strength of selection for defense can hardly be exaggerated, meaning the adaptations we talk about in this section are quite derived relative even to other social insects who share some of the same selective pressures.

NATURAL HISTORY OF DEFENSIVE BEHAVIOR AGAINST VERTEBRATES

European bees are not usually quick to sting. Often, they will first give a warning. This involves slamming into the vertebrate and making angry buzzing sounds. It is crystal clear to a person that this is a threat. If the intruder does not leave, the bees begin to sting, and because each bee leaves alarm pheromone with her stinger, the attack builds exponentially. Bees tend to go for the eyes, nose, and mouth if one is not wearing a veil, and this seems to be an adaptation for defense against furry predators. They will, however, burrow through hair and sting when they reach the skin. Even bees that have already stung the enemy will continue to harass them. These are often the most persistent bees, in fact, and many descriptions of bees chasing persons for great distances unwittingly refer to these bees.

WHY ARE BEEKEEPERS NOT STUNG MORE?

It is commonly believed, by beekeepers and bee scientists, that European bees do not sting much because of selection for gentleness. While there has been some selection for gentleness, this is unlikely the main cause for the lack of stinging. Any colony, African or European, can build to a ferocious attack under the right circumstances. It is thus worth discussing why beekeepers are not stung more as they work hives.

There are three major reasons the bee defense system fails against beekeepers. First, the use of smoke disrupts the attack, presumably by interfering with the olfactory system (Free, 1968b, Visscher et al., 1995). For a recent review of fieldwork on this topic, see Seeley (2019, pp. 82–85). Second, the importance of removable frames in keeping the bees calm cannot be overstated. When one rips comb apart, the bees become highly agitated. In nature, getting the bees' honey always involved ripping apart comb, and it is possible that they have been selected to amplify their attack when perceiving the cues associated with comb damage. Third, and most important, in a natural attack the invader goes through the entrance. That is where the guards are posted, and that is where the oldest bees (the foragers), who are probably the main defenders, are located. Getting into a wild colony's nest any other way is usually impossible because the nest is in the hollow of a solid tree. Beekeepers, in contrast, enter the nest from the top (the back of the nest) where only middle-age, food-processing bees are typically located. There has been no selection for defense in this context. Every beekeeper knows that the bees get angrier, and stings become more frequent, as one works down a hive.

Nesting Biology

The nest is central to honey bee biology, particularly in temperate zone populations, and a surprising amount of their evolution is associated with it. We review the nest's basic design and characteristics, including the properties of the famous hexagonal comb and the regulation of its construction.

Nest Characteristics

Honey bees typically nests in tree hollows, although they will nest under overhangs on cliff faces or even out in the open when cavities are not available (Seeley and Morse, 1976, Hepburn and Radloff, 1998). Seeley and Morse (1976) found a cavity size range spanning about 20 to 100 L in wild bees, with a mean of about 40 L. This size usually supports a colony of about 40,000 bees. Population size varies considerably, however, as newly established swarms may contain only a few thousand bees while the largest cavities support colonies of over 100,000 workers (reviewed in Seeley, 1985). Population size further varies with habitat and with genotype (reviewed in Winston, 1987).

Figure 10.3 shows the organization of comb in a wild nest. The brood is at the bottom while the honey is at the top. The pollen is sometimes found in a band in between, but it is also scattered about the broodnest (Camazine, 1991, Johnson, 2009a). It is important to keep in mind that a nest is composed of several combs side by side, and this representative comb is a cross section from the center. The outside combs, even at the bottom of the nest, typically contain less brood and are often used for honey storage. The comb itself is composed of three types of cells: worker cells, which make up most of the nest, and are the classic hexagonal cells known to most; drone cells, which are essentially larger versions

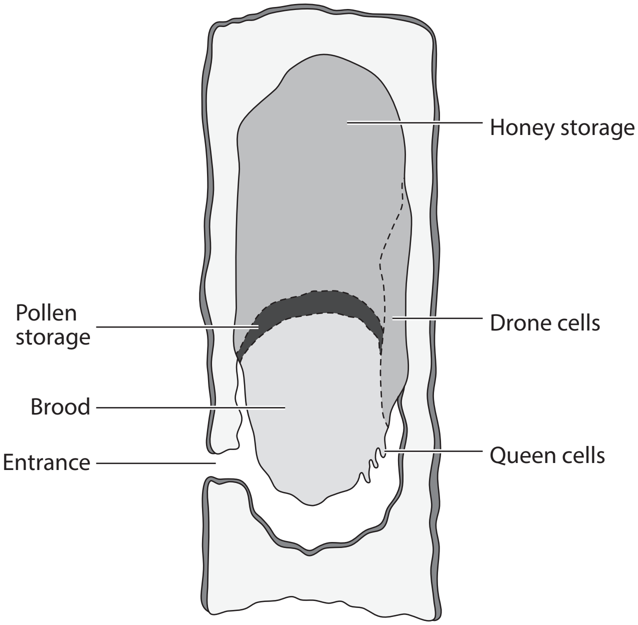


FIGURE 10.3. Organization of a wild honey bee nest (*redrawn based on Seeley and Morse, 1976*).

of the worker cells; and queen cups that hang down. Further, cells at the top of the nest, near the point of attachment to the tree, are much larger than those used to rear brood below. In general, comb used to store honey is not of uniform size, as it is drawn out as far as possible, while comb used for brood rearing is more consistent in size. An exception to this is that, with each brood-rearing cycle, comb becomes darker and the cells become smaller. This is due to the incorporation of the pupal cocoon and waste material into the wax after bee emergence. In old brood comb, the cells are thus black and small and the bees that emerge from them are smaller than average (Abdellatif, 1965, Winston, 1991). Honey combs tend to maintain their light color through time.

Comb Construction and Regulation

COMB BUILDING

Middle-age bees (MABs) are the temporal caste in between nurses and foragers (Johnson, 2008b). They perform all within-nest activities other than nursing (see chapter 12 for details). It is these bees that activate their wax glands to produce wax flakes (plate 9), which they manipulate with their legs and mandibles for construction (reviewed in Winston, 1991). The wax glands are on the ventral side of the bee abdomen and are associated with the fat body (Cassier and Lensky, 1995). These glands are inactive in nurse bees and are atrophied in foragers, as is typical for glands that underlie a task associated with the temporal polyethism system (Boehm, 1965, Johnson, 2010a). With respect to the process of construction, the building bees form a cluster, which warms itself up to about 40°C, the optimal temperature for manipulating wax (Darchen, 1991, Pirk et al., 2004a). The wax flakes are then mixed with saliva and propolis. New comb begins as a strip of wax, but it is quickly drawn out into the characteristic hexagons after a brief circular phase (Karihaloo et al., 2013). This hexagonal shape is common to the brood-rearing cells of many social insects, as it leads to the maximum number of cells within the minimum amount of space.

Exactly how bees build the hexagons has long been a subject of inquiry. Initially, it was assumed that bees shape the comb like a human sculpture and thought went into how they might do this (reviewed in Winston, 1991). Then it was suggested that the process may be simpler and dependent on the physics of heating the wax. Specifically, heating up the wax to a liquid state was argued to trigger a self-organizing transition into hexagons from the initial circular shape of newly built cells (Pirk et al., 2004a). Later work, however, did not support this model, as it recorded temperatures during building too low for that to occur and instead supported a process of more direct building (Bauer and Bienefeld, 2013, Nazzi, 2016). The question remains unresolved.

REGULATION OF COMB CONSTRUCTION

Comb is composed of wax, a lipid, and is costly to make. It likely takes at least six times a given quantity of honey to make the same weight of wax (Whitcomb, 1946, Weiss, 1965). Hence, wasted wax production would be a major drain on colony resources. Thus, bees must construct as much wax comb as is necessary and no more. How bees solve this problem is poorly understood, although a good start has been made.

Pratt showed that the initiation of comb building correlates with a threshold level of (1) comb fullness and (2) a sufficient amount of nectar flowing into the hive (Pratt, 1998a). This makes sense, as there is no need to build new comb if enough is available and there is no need to build when nectar is not flowing. These correlations were tested experimentally by altering the amount of free space in nests, and the rate of nectar intake, and recording building activity. It was shown that colonies only start comb building if both conditions are met simultaneously. Once comb building starts, however, all that is necessary to keep it going is the continuation of the honey flow. This makes sense, as honey flows can be quite dramatic, and as long as the nectar continues to be available more space will be necessary.

A related question is how the colony balances the ratio of worker-to-drone comb. Oster and Wilson (1978) in a classic text argued that social insect colonies are similar to plants in that they invest in growth alone until they reach a given size and then they switch to balancing their efforts between growth and reproduction. This might suggest that drone comb production, which limits the maximum rate of worker production, would be postponed until a threshold colony size is reached. Recent work on the establishment of swarms of variable size supports this notion (Smith et al., 2014, 2016). Oster and Wilson's idea also proposes that an adaptive ratio of investment between worker and drone comb should happen in fully mature colonies, and work by Pratt supports this hypothesis. Addition of drone comb to a colony leads to less drone comb building, while removal of drone comb triggers more building (Pratt, 1998b). This suggests that the ratio is regulated. The mechanism by which this is accomplished is unknown, but Pratt's work suggests that direct assessment of the colony's state may be involved. MABs, the relevant caste, spend at least 25% of their time patrolling the nest inspecting cells (Lindauer, 1952, Johnson, 2008b). They thus have ample opportunity to directly assess colony state (Johnson, 2008a).

Propolis

When bees move into a new tree cavity, it is often lined with rotting wood. The bees use their mandibles to strip this soft wood away and then apply a thick layer of propolis, tree resin, to the hard wood beneath (Seeley, 2019). Bees also use propolis to fill holes in the tree cavity to aid in thermoregulation. Bees collect propolis from many types of trees, so propolis varies in color, consistency, and antimicrobial properties across geographical regions (Bankova et al., 2018, 2019). Recent work has shed light on the adaptive benefits of propolis use in the nest. Colonies living in Langstroth hives given a propolis envelope inside the box, to mimic what is found in nature, had

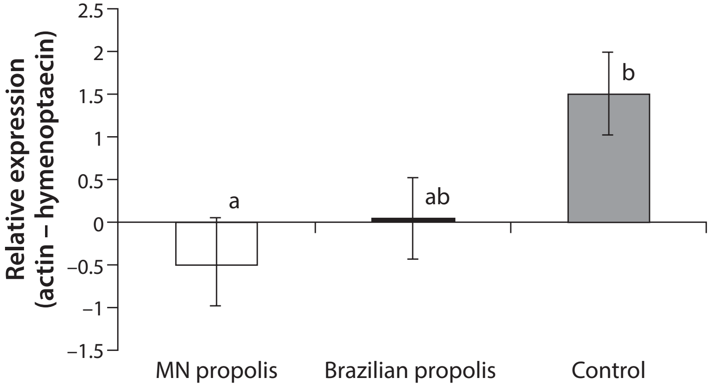


FIGURE 10.4. Transcript levels of the antimicrobial peptide hymenoptaecin in colonies with and without propolis envelopes (*from Simone-Finstrom et al., 2017*).

bees with less activated immune systems than bees living without an envelope, as shown in figure 10.4 (Simone et al., 2009, Simone-Finstrom and Spivak, 2010). A propolis envelope has also been shown to decrease levels of various pathogens in the nest (Borba and Spivak, 2017, Simone-Finstrom et al., 2017).

11

The Honey Bee Colony Is a Superorganism

There is probably no more contentious issue in social insect biology than the utility of the superorganism concept (Wheeler, 1911, Emerson, 1939, Wilson, 1971, Seeley, 1989a, 1997, Moritz and Fuchs, 1998, Reeve and Hölldobler, 2007, Hölldobler and Wilson, 2009, Johnson and Linksvayer, 2010, Haber, 2013, Helantera, 2016, Boomsma and Gawne, 2018). Although it has many proponents, there are also researchers, and particularly philosophers, who think the concept is a distraction (Mitchell, 1995, Ghiselin, 2011). In this chapter, we attempt to clear up several misconceptions about the superorganism concept and explain why so many honey bee biologists view it as an essential idea for understanding colony organization.

Metaphors versus Convergence

Traits between species can be shared either because they were inherited by common descent, in which case they are called homologous, or because natural selection produced similar adaptations in organisms not related by descent, in which case they are called analogous. The reason independent selection processes converge is rather obvious. When faced with the same problem, selection often comes up with the same (or similar) answer. The shared body shape of dolphins and sharks, the similar wing design of birds and bats, and the same farming symbioses of ants or termites with fungi are all classic examples of convergent evolution. The superorganism concept is also an argument based on convergence in form and process between metazoan bodies and insect colonies.

In contrast to supposing the similarity between an insect colony and a metazoan body is due to convergence, it is often said that this similarity is simply metaphorical (Mitchell, 1995, Ghiselin, 2011). The problem with this criticism is that the authors who dismiss the superorganism concept as a metaphor do not explain what they mean by *metaphor*. A metaphor is a concept that can have multiple meanings. The most common usage is to say that one thing is like another in order to get some point across. When Shakespeare wrote that the world is a stage, he meant that life is like a play in that we are always acting. He did not mean that life is literally a play. Likewise, if one says that some human dictator is the silverback gorilla, or the matriarch elephant, this is also a metaphor. However, it is a different kind of metaphor because these are all leaders. They are literally the same thing in at least some sense. In short, metaphors can be comparisons between things that are only poetically similar, or they can be comparisons between things that are functionally similar. In the second case, which is relevant here, the crux of the matter is how and why the different things are similar, and to what extent.

To focus on a biological case of functional similarity, when one says that a bird's wing is like a bat's wing, one is not using the word *like* in a poetic, metaphorical sense. One is saying that in both contexts the problem is one of achieving flight, which involves generating lift and thrust, and that selection converged on the same solution in both clades: a wing. When exploring flight mathematically, for example, the same equations (reparameterized) are sometimes useful in both cases because the same principles are at play. The same equations can even be useful (to an extent) when studying aircraft with fixed wings. Hence, knowledge of any one of these systems can guide (with caution) explorations of the others. Essentially, there is not a metaphorical similarity between the different mechanisms of flight in birds and bats; rather, they offer different but conceptually similar solutions to the same problems. In such cases, a common terminology is appropriate for describing analogous adaptations.

To turn to social insects, when one says that a social insect colony has an anatomy and a physiology, this is not a poetic metaphor. Rather, one is saying that selection has resulted in the same organizational structures and processes in both insect colonies and metazoan bodies. In both cases, the problem that selection solves is to maximize the reproductive success of a group of many interacting individuals. Division of labor, which is at the core of what is meant by anatomical specialization in the case of a honey bee colony, increases output, but it can lead to lack of unity of purpose at the group level; thus, there is a need for intense communication between the specialized parts. This is the essence of physiology. Hence, the superorganism idea is a logical and useful conceptual model for understating social insect colonies.

A Practical Superorganism Concept

There have been many formulations of the superorganism concept through the years. The idea was proposed by Wheeler (1911), for example, and his work stressed an analogy between gonads and somatic cells on the one hand and social insect queens and workers on the other. Emerson (1939) later realized that the analogy extended to physiology as well. Later formulations were made by several authors (Seeley, 1989a, 1997, Moritz and Southwick, 2012, Hölldobler and Wilson, 2009, Johnson and Linksvayer, 2010, Helantera, 2016). There is not space here to review this large body of work, but it is referenced so that the interested reader can investigate it. The version of the superorganism concept we use here mainly grows from Tom Seeley's work (Seeley, 1989a, 1997). It was given its present form, however, in Johnson and Linksvayer (2010).

Social Anatomy

The central adaptation within an organism, or a superorganism, is division of labor (DOL). In our bodies, this takes the form of specialized organs. In a social insect colony, this can take the form of either physical castes (morphologically specialized individuals, like queens and workers) or temporal castes. In both cases, tasks are divided between groups, and this DOL leads to greater productivity, usually due to specialization effects. When an individual devotes itself to a a subset of tasks, it can perform them better (Jeanne, 1999). This is sometimes due to physiological or morphological specialization, but it can also be due to the logistics of the work being conducted. For example, a honey bee forager who finds a rich, but ephemeral, nectar source, can make many trips to it if she specializes on collecting nectar. But if she were to both collect nectar and then take time to process it into honey, she would not be able to maximize her rate of visits to the food source.

Social Physiology

Social physiology is a term coined by Seeley (1995) to refer to the mechanisms whereby the members of a social insect colony coordinate their activities at the group level. A key point is that this idea did not grow out of armchair philosophizing about how a colony should work. It grew out of decades of research. This is lost in many discussions of the superorganism concept. We first consider this idea in broad terms, with an analogy to humans, which often face similar problems. We then give some insight into the nature of social physiology in insect colonies.

DOL increases productivity, but it creates a potential for lack of coordination between the parts of the system, which can decrease its productivity. To draw an analogy, suppose a group of people are building a brick wall. They may decide to use DOL and break into three specialized groups: the brick layers, the mortar mixers, and the laborers who carry the bricks and mortar from where they are stored/prepared to where they are used. To make this system work, there must be communication and coordination between the groups. If too many people are laying bricks and not enough are acting as laborers, then the brick layers will waste time standing around waiting for mortar and bricks. In short, an optimal (or at least functional) number of workers in each task must be achieved and then maintained. Further, even if a given number is best in the morning, there is no guarantee that it will be optimal in the afternoon when it is hotter and the brick laying is more difficult. There must be continuous updating to maintain a shifting optimal ratio.

That DOL in insect colonies requires social physiology has been demonstrated in work by Robert Jeanne on social wasps (Jeanne and Nordheim, 1996, Jeanne, 1996, 1999). He compared and contrasted the organization of the work between small, independent nest founders, such as *Polistes* spp., and large, swarm founding wasps, specifically, *Polybia occidentalis*. He sought to understand why the small-colony species do not use assembly lines, while the large-colony species do. They focused on nest building. *Polybia* species build a paper wall (carton) around their combs for defense, and the workers are easily observed during the construction process. Jeanne and collaborators found that, in *P. occidentalis*, wasps either specialize on pulp collecting, water collecting, or carton building. They also found that there are specialization effects that make DOL adaptive (wasps can carry heavier loads of pulp or water when specialized), but that if too few wasps participate (less than about 50), then the costs of queuing delays outweigh the benefits of DOL. Examples of queuing delays are when a building wasp has to wait around for more pulp or a water collector has to wait to unload to a builder before returning to her water source. When everyone works independently, these wait times do not happen. They also do not happen, to an appreciable extent, with a large number of workers. It is only in the context of small numbers specialized on each task that they lead to much wasting of time. Hence, small colonies cannot use an assembly line (as the whole colony is fewer than the number needed for just one partitioned task), but large colonies can and do. Jeanne and his coworkers further showed that, when the assembly line is in place, there is a nonlinear increase in per capita work output with increases in colony size. This explains why assembly lines evolve and come to dominate organization of work in large societies. Jeanne was not able to determine how the wasps coordinate the allocation of workers to the three specialized groups, however. This is where honey bees become relevant because we know more about how they coordinate work across activities.

Much of Seeley's book *The Wisdom of the Hive* (1995) pertains to how foragers coordinate their activity with middle-age bees (MABs) in the process of making honey. The foragers collect the nectar and the MABs receive it, process it into honey, and build the storage space for it. The efforts devoted to the three tasks must be coordinated to achieve efficient functioning at the group level. If too few nectar processors are working, for example, then the nectar foragers cannot unload their nectar and foraging is hampered. Likewise, if nectar storage space runs out, then nectar foraging and nectar processing shut down. Accordingly, when Seeley experimentally removed nectar receivers, foraging shut down (Seeley, 1989b). The foragers, however, did not stand around and wait for things to improve. Instead, they quickly began to produce signals to fix the problem. One such signal is called the tremble dance (Seeley, 1989b, 1992b, Seeley et al., 1996). The nectar foragers left the dance floor area (just inside the nest entrance), where these bees typically remain, and they walked all around in the nest, producing the tremble dance. Essentially, they broadcast a message to the entire colony (Wilson and Hölldobler, 1988). Seeley found that, soon after they did so, many more workers started functioning as nectar receivers and then the nectar foraging would resume. He hypothesized that nectar foragers use a simple rule based on the wait time to find a nectar receiver to know whether there is a balance of effort between nectar foragers and nectar receivers. A short wait time (indicating a balance) stimulates nectar foragers to continue their work and to perform waggle dances (to activate more foragers), while a long wait time causes them to stop foraging and to produce the tremble dance. Evidence supports this idea (reviewed in Seeley 1995), although the tremble dance perhaps has other functions as well.

Applications of the Superorganism Concept

We now consider how research can go wrong when a superorganismal approach is not taken. We focus on two issues. First, because a colony is an organism, in vitro versus in vivo problems occur. This problem is so common, in fact, that this should be made clear to all students of social insect biology, and efforts to avoid it should be mandatory for major publications. Second, activities that are organized at the individual level in solitary insects are often distributed across individuals in insect colonies, so the same approach to their study will not work in both cases.

In Vitro versus in Vivo Errors

When cells are grown in tissue culture, they are separated from the dense network of communication signals that tie these cells into an organism. A consequence of this is that, although cells may behave with regularity in an artificial container, this regularity may have little to do with how they function in a body. This is widely known in molecular biology. The same problem occurs repeatedly in studies of social insects and for the same reason. A worker is embedded in a dense network of interactions that guide her physiology and behavior. Studies of workers outside their natural social setting are thus like studies of cells in tissue culture.

One example of an important question that was addressed in a manner that led to an in vitro problem relates to kin recognition. When worker honey bees were reared in isolation and then given a choice to associate with close or far kin, they choose the near-kin individuals, statistically speaking (Getz and Smith, 1983). This was interpreted to mean that workers can distinguish full sisters from half sisters, thus setting the stage for much nepotistic conflict within a colony. However, when the same experiment was conducted with bees that were reared in groups, or in a nest, the preference for associating with kin disappeared (Getz and Smith, 1986). It is now known that a gestalt mechanism operates in nestmate recognition such that everyone in the nest shares their labels to achieve one colony-level identity (Breed et al., 1998, van Zweden et al., 2010). Thus, although bees can recognize kin when reared in isolation, this result is an experimental artifact. In general, any result found with bees in vitro (in a lab bioassay) must be verified in vivo (in a real colony) because important colony-level inputs are missing in small, artificial groups.

It is important to close this discussion with a final point. The argument being made is not that cage studies, or other simplified bioassays, are useless. These approaches are useful, just as tissue culture studies are useful in molecular biology. It is, rather, the case that the results of studies conducted using highly artificial settings, no matter how persuasive, are preliminary until verified in whole colonies. This is recognized in molecular biology, where publishing work based entirely on tissue culture work is frowned upon; unfortunately, this problem is not yet widely appreciated in social insect biology.

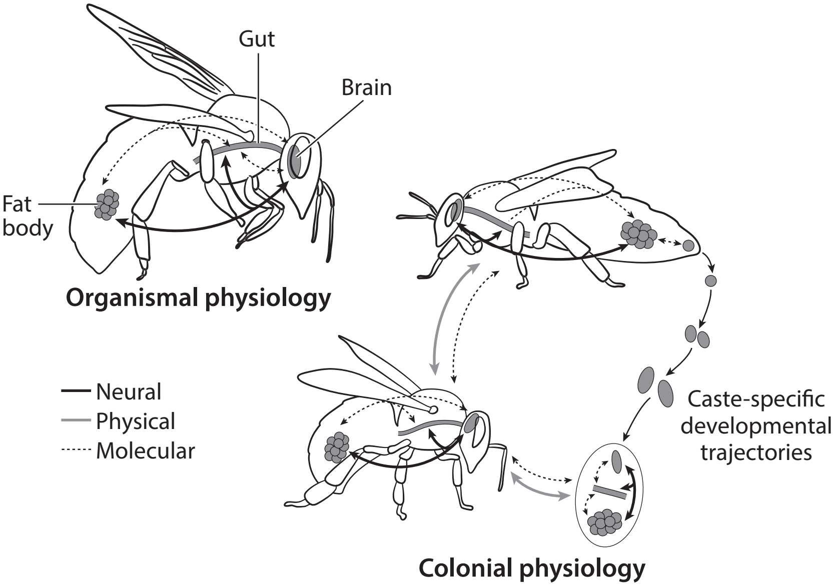


FIGURE 11.1. Major transitions in evolution involve the transfer of control mechanisms from one level of organization to another. In the case of the superorganism, many regulatory processes guiding development and behavior, which are based on endocrine signaling in solitary insects, are based on exocrine signaling between individuals in colonies (*redrawn based on Friedman et al., 2020*).

Distributed Physiology

The superorganism is characterized by processes that were once controlled at a lower (individual) level of organization being shifted to a higher (colonial) level of organization. When a solitary insect gets hungry, for example, it forages for food. Honey bees, however, store vast quantities of food, and individuals do not typically experience hunger. Rather, honey bees forage whenever nectar is available or when the colony needs more pollen (Fewell and Bertram, 1999, Calderone and Johnson, 2002, Sagili and Pankiw, 2007). Likewise, the signals for regulating ovary development in a solitary insect come from the brain and endocrine system. These systems are still present and serve the same functions in honey bees; however, they are now downstream of signals from the queen and brood (figure 11.1). Hence, the network controlling ovary activation is considerably larger in honey bees than in solitary bees and is distributed over multiple castes (Linksvayer et al., 2009a, 2009b, 2011). One could go on with these examples. The take-home message is that many traits are distributed over a network of individuals (Wilson and Hölldobler, 1988). The gene networks controlling many traits are thus correspondingly larger and more complex in social than in solitary insects, so one cannot simply apply the research approach used for *Drosophila* in the more complex systems of social insects (Johnson and Linksvayer, 2010, Friedman et al., 2020).

Society versus Organism

There are two worldviews in the study of insect societies. The first is that these are animal societies—more complex than lion prides but similar in principle. The second is that these are organisms. The types of questions that are asked and how they are explored will differ in relation to which worldview underlies a research program.

By the first view, an insect society is composed of independent agents that are totipotent organisms. They interact with one another, but the overwhelming majority of their behavior is individual oriented. They may engage in communication, for example, but the conflict of interest between sender and receiver leads to much dishonesty, so mechanisms ensuring honest communication are paramount. This "society view" is appropriate for some species of social insects, such as paper wasps (*Polistes*).

By the second view, an insect society is a single agent with many parts. An individual in this situation is thus like a cog in a machine, and one can only understand how each cog works by interpreting their behavior in reference to colony-level goals. Further, the overwhelming majority of communication is honest in this situation. The dance language system, for example, and many other colony-level processes, are based on honest communication between individuals with a common goal. This guides research toward understanding the connections between individuals, and to colony-level signaling mechanisms. It further forces one to take seriously the fact that many processes and traits that occur at the individual level in other animals are distributed across many bodies in an insect society.

12

Division of Labor

Aristotle first discussed honey bee division of labor (DOL) in writing, but it was Huber in the 1700s who first described it (Huber, 1792). The modern study of DOL began perhaps with Rosch, a student of von Frisch's (Rosch, 1925, 1927, 1930). He described the basic temporal polyethism system that is the main subject of this chapter. Today, behavioral studies on DOL in honey bees continue, but most of the field now focuses on the underlying genetics and endocrinology. We start with a review of the behavioral work, before turning to the molecular studies.

Caste and DOL

DOL in the broadest terms refers to individuals having biases (weak or strong) in their task choices (Oster and Wilson, 1978, Page and Robinson, 1991). The concept of a caste (morphological or temporal) is associated with strong DOL (Wilson, 1976, Seeley, 1982, Wilson and Hölldobler, 1988, Hölldobler and Wilson, 1990, Johnson, 2010a). In such cases, the individuals who share the same task repertoire are called a caste. Under normal conditions, castes do not perform tasks outside their task repertoire. Further, even in cases in which caste members can perform tasks outside their task repertoire, they tend to perform them poorly (Vance et al., 2009, Chang et al., 2015). Castes can be based on physical differences between workers, as in the reproductive castes of honey bees, or on developmental differences in physiology, which are typically associated with an age-based caste system.

Reproductive Division of Labor

The central DOL in an insect colony is between the queen and workers (Wilson, 1971). In simpler societies, the queen plays many roles in the nest, such as guarding, engaging in dominance interactions with the workers, and some-

Table 12.1. Honey bee tasks

Nurse-specific tasks
Feed brood
Eat pollen
Attend queen
Cap larval cell
Messenger bee activity

MAB-specific tasks
New comb building
Nectar processing: Receive nectar
Nectar processing: Tongue lashing
Bee bread production: Pollen packing
Guarding
Undertaking
Hygienic behaviorWashboarding

Forager-specific tasks
Water collection
Pollen collection
Nectar collection
Propolis collection
Scouting
Nest assessment
Nest defense
Waggle dancing
Tremble dancing
Producing shaking signal
Worker piping

Caste-independent tasks
Stand
Walk
Inspect cell/patrol
Work wax/repair comb
Trim brood/honey cappings
Self-groom
Trophallaxis
Head in honey cell
Head in empty cell: Nest heating
Work propolis
Fanning
Allogrooming

times caring for the young (Schwarz et al., 1998, Saleh and Ramirez, 2019). In the most derived insect societies, like the honey bee, the queen is little more than an egg-laying machine. She is missing key morphological structures necessary for many tasks, and when laying eggs she cannot even fly. Hence, reproductive DOL is simple in honey bees. The main activities that the queen engages in, other than egg laying, relate to reproductive signaling, which is covered in chapter 14 on chemical ecology.

Temporal Polyethism or Temporal Castes

In honey bees, workers pass through distinct age castes that specialize in different activities, as shown in table 12.1 (Seeley, 1982, Calderone, 1995, Calderone and Page, 1996, Johnson, 2003, 2008b). We first discuss the data

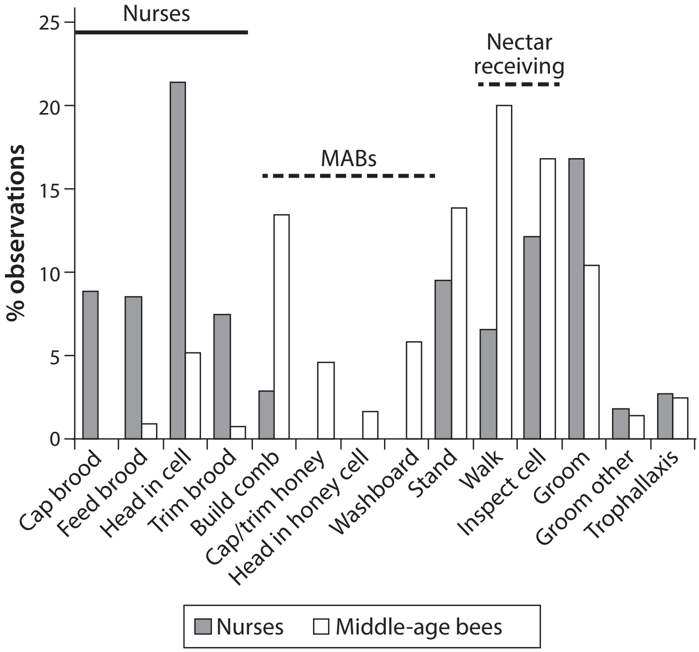


FIGURE 12.1. Task performance rates based on focal animal observations of nurses and middle-age bees. Classic MAB tasks are indicated by dashed lines, one of which appears above those tasks that are part of nectar receiving (*from Johnson, 2010a, based on Johnson, 2008b*).

supporting this notion in general and then describe in detail the behavior of each caste. In a classic study, Seeley (1982) followed a cohort of age-marked bees with scan sampling. These data suggested four temporal castes: cell cleaners, nurses, middle-age bees (MABs), and foragers. Although the basic temporal progression was clear in this study (and in other older datasets), the degree of discretization of the task repertoires was unclear. On day 12 of life, for example, it could be the case that a fraction of bees are either nurses or MABs, or it could be that bees this age split their time between these activities. Two studies by Johnson explored this matter. First, it was shown that there is little to no overlap in the task repertoires of nurses and MABs when followed with focal animal observations rather than scan sampling to improve resolution (figure 12.1) (Johnson, 2008b). A strong distinction between the behavior of the two tasks is associated with movement. MABs

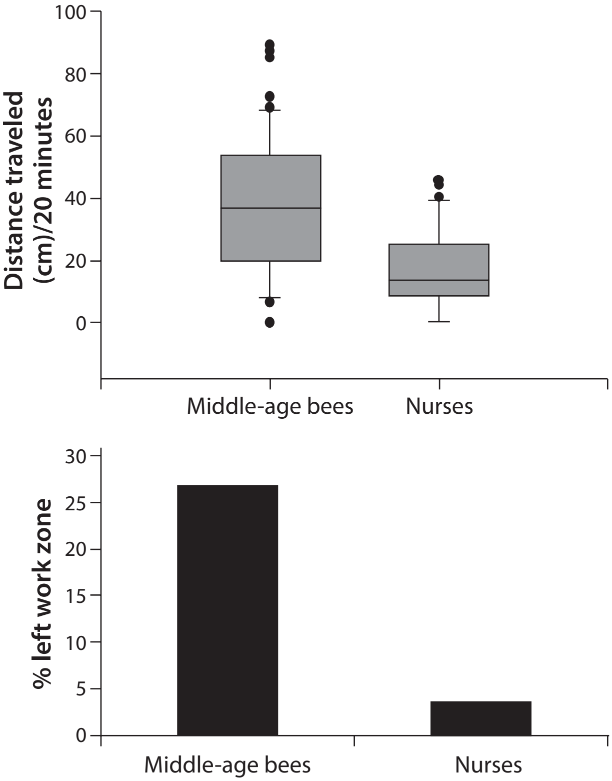


FIGURE 12.2. Movement patterns distinguish nurses from middle-age bees. Top: Distance traveled over 20 minutes in groups of nurses and middle-age bees. Bottom: Percentage of bees leaving the work zone in which observations began for nurses and middle-age bees (*from Johnson, 2008b*).

conduct patrols, which take them all over the nest, while nurses rarely leave their work zone (figure 12.2). Second, when the study of Seeley (1982) was repeated with observations of individually marked bees (as opposed to bees marked only for age), it was discerned that the majority of bees pass from one caste to the next with little mixing of caste-specific task repertoires (Johnson and Frost, 2012). The overlap in the task repertoire of 12-day-old bees, for example, is thus the result of some 12-day-old bees being nurses while others are MABs, and not because 12-day-old bees split their time between these task sets.

CELL CLEANERS

Newly emerged bees have soft exoskeletons and therefore cannot fly or sting (plate 10). Their development is still incomplete, and for the first few days of life they do little but feed (both in pollen cells and by receiving trophallaxis from nurse bees) and continue their development (reviewed in Johnson, 2010a). They stay in the warm broodnest, which presumably aids in their development. The only task they perform is cell cleaning. The cells of newly emerged bees contain the discarded pupal cocoon and waste materials, and the newly emerged bees spend their time cleaning these cells. They do not necessarily clean their own cell, however, as after emergence they tend to meander off, grooming themselves, and other bees quickly clean their cell. This phase of the temporal polyethism system is the least variable, as bees spend about three days in this caste.

NURSES

Nurse bees have well-developed hypopharyngeal glands (HPGs) that they use to produce brood food, along with secretions from the mandibular glands (Haydak, 1963, 1970, Kubo et al., 1996, Sagili et al., 2005, Pankiw et al., 2008, Wegener et al., 2009). Nurses are often thought of as feeding the young, but it is important to realize that they are the major source of protein and lipids for all the bees in the nest (Crailsheim, 1990). This includes the queen and the older workers. It has been estimated that about half of the brood food (depending on the amount of brood in the colony) goes to the larvae, while the other half goes to the workers (figure 12.3) (Crailsheim, 1991, Crailsheim et al., 1992). The nurses also spread queen mandibular pheromone around the nest, in their role as messenger bees (Seeley, 1979). Nurses further work wax within the brood zone. This involves recycling the wax previously used as caps after newly emerged bees leave their cells and, most importantly, capping larvae ready to begin pupation. Although middle-age bees also work wax within the brood zone, they do not cap larvae, suggesting that it is only the nurses who acquire the relevant information necessary to make this decision (Johnson, 2008b). Another major nurse bee activity is trimming cappings. Bees are very frugal with their wax. After a cell is capped, the wax in the capping is trimmed as thinly as possible with the excess wax being used elsewhere. This is a very time-consuming task that is done by both nurses and MABs. With respect to location, the nurses rarely leave the brood zone. If they do, it is only

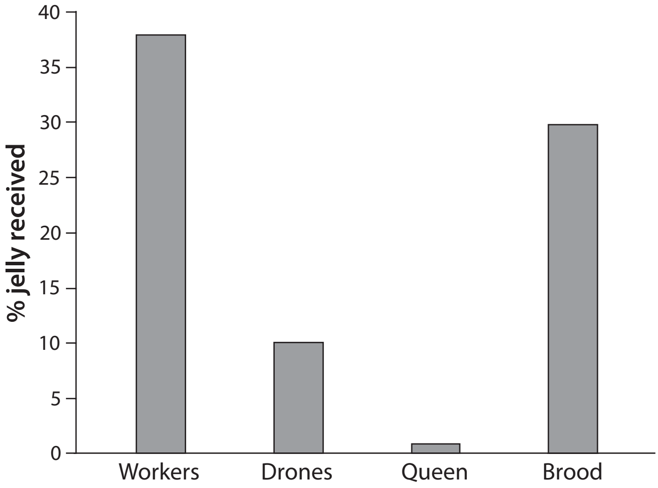


FIGURE 12.3 Nurses split the brood food they produce between all members of the colony (*redrawn from Crailsheim, 1991*).

to find pollen cells. Finally, nurses, in addition to all the other castes, also engage in tasks that are not associated with age, such as thermoregulation and CO_2 regulation (Johnson, 2008b).

MIDDLE-AGE BEES (FOOD PROCESSORS AND BUILDERS)

Middle-age bees fall between the nurses and the foragers in the developmental sequence (Johnson, 2008b). These bees have two main activities: building the nest, which sometimes involves secreting wax, and processing food. MABs also conduct myriad other tasks, however (table 12.1) (Seeley, 1995, Trumbo et al., 1997). With respect to building, the wax glands of MABs secrete wax scales, which the bees moisten and work into comb (reviewed in Winston, 1991). As with nurses, however, most wax working involves trimming excess wax from here and there to use elsewhere, not building new comb. Food processing involves receiving nectar from the foragers at the bottom of the nest and transporting it to the top of the nest, where a behavior called tongue lashing is used to lower the water content and turn it into honey (Seeley, 1989b, 1995). MABs also process pollen into bee bread, another long-term food storage product. Other tasks involve working propolis, washboarding (smoothing rough surfaces), guarding the entrance, removing dead bees, and hygienic behavior (uncapping and removing dead larvae). A major unresolved question with respect to MABs is whether there is another layer of specialization within this group. Pratt (1998a), for example, found that the nectar receivers are not comb builders. Hopefully, future work will address this question.

FORAGERS

Foragers only perform two within-nest tasks. They help with thermoregulation when it gets too hot, and propolis collectors help with working this material in the nest. Otherwise, foragers focus on outside activities: foraging for food and other substances needed by the colony and defense. With respect to foraging, foragers collect four different substances: nectar, pollen, water, and propolis (plates 11–12). In general, most foragers are collecting nectar at a given time, but pollen foraging can periodically be higher depending on colony needs (Seeley, 1995). In general, colonies collect as much nectar as they can and only as much pollen and water as they need (Fewell and Winston, 1992, Pankiw et al., 1998b, Dreller et al., 1999, Calderone and Johnson, 2002). They tend to collect a constant, but small amount of propolis through time (Nakamura and Seeley, 2006).

Atypical Caste Patterns

Some bees begin to forage at a young age. These bees are called precocious foragers (Page et al., 1992, Huang and Robinson, 1996, Jassim et al., 2000). A small number of such bees are a constant, but their numbers increase when the forager caste is removed from a colony. This shows flexibility in how bees move through the temporal polyethism system (Robinson, 1992, Huang et al., 1998). Further, when the young bees are all removed from a nest, some foragers revert to being nurses (hereafter called reverted nurses) (Huang and Robinson, 1992, 1996). Hence, it is possible to decouple age and caste. Reverted nurses and precocious foragers are invaluable for understanding the mechanistic basis of caste-specific behavior and physiology. They are also likely important to colony function after swarming, a time when such transitions occur naturally (Sakagami and Fukuda, 1968).

Although reverted nurses and precious foragers provide insight into the mechanisms underlying caste transitions, it is important to stress that inflexibility has also been found regarding caste switching. Perhaps the key point to make regarding reverted nurses and accelerated foragers is that they are not terribly good at their jobs. Most larvae die when the nurses are removed, as too few foragers revert to care for them and those that do have small HPGs relative to normal-age nurses (Huang and Robinson, 1996). Accelerated (young) foragers also take fewer foraging trips per day, carry smaller loads, and get lost and do not return as often from the field relative to normal-age foragers (Vance et al., 2009, Chang et al., 2015, Perry et al., 2015, Ushitani et al., 2016). Further, although the colony is flexible, the flexibility is less than is sometimes assumed. When Johnson (2005) removed the foragers from a nest and then compared foraging in that nest relative to control colonies of the same size, the colonies that lost their foragers foraged at greatly reduced rates for over two weeks. This was likely due to too few foragers overall and the small number of precocious foragers taking fewer trips.

Mechanistic Studies of DOL

The key to understanding the proximate basis of DOL relates to determining how evolution has crafted novel developmental patterns from ancestral pathways (West-Eberhard, 1987, 2002, Robinson et al., 2005, Amdam et al., 2006a, Johnson and Linksvayer, 2010). This involves both the study of changes to conserved pathways and the study of novel genes and pathways. These themes pervade the rest of this chapter. To ground this discussion, we start with a quick overview of some of the basic principles at play. Each caste has a distinct task repertoire and a physiological state specialized for the performance of those tasks (Robinson, 1992, Johnson, 2010a). Hormonal, and gene expression, changes are associated with each change in caste to coordinate these physiological and behavioral changes (Huang et al., 1994, Rutz and Luscher, 1974, Cash et al., 2005). Essentially, the biology of this phenomenon is such that evolution has taken the basic hormonal and physiological mechanisms that underlie the distinct behavior of juvenile and adult forms in solitary organisms and elaborated them to control caste. Honey bees, in a sense, go through puberty three times and exhibit specialized behavior during each developmental phase, or caste, that fits into a larger colony-level system of DOL.

Physiological Studies

Most issues in physiological specialization can be thought of in terms of (1) the specialized glandular and other tissue-specific physiology that is necessary for optimal task performance, and (2) the endocrinological mechanisms controlling specialized physiology. We start by covering work on nutrition and DOL, however, as it does not fit well into either paradigm.

NUTRITION

A series of studies showed that nutritional stores and hunger are associated with the control of DOL. Schulz et al. (1998) showed, using single-cohort colonies, that bees from starved colonies foraged earlier than did bees from colonies with food stores. Toth et al. (2005) then showed that foragers have lower lipid and protein stores than nurses and that the low lipid stores are evident prior to the commencement of foraging. In a follow-up study, they showed that interrupting lipid synthesis with a fatty acid inhibitor induces precocious foraging (Toth and Robinson, 2005). It was further shown that bees reared in isolation have low lipid stores from a young age, suggesting that social interactions in the colony control lipid stores.

There are two ways to interpret these data. First, biological traits are complex such that there is often a plan A and a plan B. If we were studying parental care in leopards, for example, and did an experiment in which we took away the mother and showed that the cub tried to forage, would that show that parental care is not of paramount importance because the young can fend for themselves? Of course not; it would mean there is an emergency set of behaviors that the young will engage in when absolutely necessary, which are probably not associated with much success. It is the same for many honey bee traits. If young bees are starved because no food, or old bees, are in the nest, they will go out and forage, but they are not suited to it and have little success. Further, in a healthy nest there are always food stores, meaning that DOL typically proceeds without hunger entering the equation.

The second, more important, way to interpret the work on nutritional stores is to hypothesize how it fits into a model for control of DOL. It may be the case that when bees leave the nursing caste they are no longer stimulated to feed on pollen (probably due to lack of interaction with key primer pheromones), and this triggers the loss of lipid and protein stores (Johnson, 2010a). This would suggest that the pattern of falling nutrient status should begin at the transition to the middle-age caste. This is speculative, but hopefully future work will test this idea.

GLANDULAR AND OTHER TISSUE-SPECIFIC SPECIALIZATION

Many tissues change in function at caste transitions in order to specialize the bee for her new role in the nest. This is clear from large-scale gene expression differences found between nurses and foragers across most tissues (Jasper et al., 2015). Among the many tissues, the HPGs are the best understood. These glands produce brood food in nurses and nectar processing enzymes and antimicrobial peptides (AMPs) in foragers (Knecht and Kaatz, 1990, Huang et al., 1994, Vannette et al., 2015). It is unclear what this gland does in MABs. The mandibular gland also strongly changes in function, with increased expression of AMPs and genes associated with detoxification and immunity in foragers (Vannette et al., 2015). Some glands do not change in function, however; they simply turn on or off at the right time. The venom gland is initially not active, for example, but once it turns on, it stays active (Owen and Bridges, 1976, Owen and Pfaff, 1995). Much work has explored changes in fat body function, which produces vitellogenin (Vg) when the bees are nurses (Amdam et al., 2003a, 2005b, 2006b, Ament et al., 2010). Changes in digestive physiology between castes have not received much attention, but based on transcriptomic work there are massive differences in digestive enzyme expression between nurses and foragers (Jasper et al., 2015). This may relate to the nurse's role as the source of protein and lipids for the whole colony, which entails eating pollen, something older bees never do. Thoracic muscles also show large changes at the transition to foraging that optimize their physiology for flight (J. B. Williams et al., 2008). Finally, major changes in brain structure and function occur at the transition to foraging (Fahrbach et al., 1995, 1997, 2003).

ENDOCRINOLOGICAL MECHANISMS

JH and DOL. Juvenile hormone (JH) is a sesquiterpenoid that governs many aspects of insect development, behavior, and physiology (Nijhout and Wheeler, 1982, Jindra et al., 2013, 2015). For most adult insects, JH is high in the young and low in older reproductive individuals (Nijhout, 1994). Early studies in honey bees showed that JH increases with age as bees progress through the caste system (Jaycox et al., 1974, Rutz et al., 1976, Fluri et al., 1982, Robinson, 1987, Huang et al., 1991, Robinson et al., 1991), the inverse of what is seen in solitary insects. Treatment with JH, or methoprene, a JH mimic (via injection or topical application), was then shown to cause inhibition of HPG development and an early transition to MAB tasks and, consequently, precocious foraging (Jaycox et al., 1974, Jaycox, 1976, Rutz et al., 1977, Fluri et al., 1982, Robinson, 1985, 1987). Later studies showed that JH is quite low in nurses and young MABs, before growing higher in older MABs and foragers (Huang et al., 1991), an improvement on the temporal resolution shown in earlier studies. Later work by Sullivan et al. (2000) showed that bees with their corpora allata (CA) removed (the CA produces JH) foraged later than did sham-operated control bees.

Some important conclusions about JH and DOL can be drawn from these studies. Fundamentally, DOL is not controlled in a simple manner by JH, or probably any other endocrinological mechanism. This is clear, given that there are sharp behavioral differences between nurses and young MABs but little to no difference in JH titer (Huang et al., 1994, Johnson, 2008b). The same is true for older MABs acting as guards or undertakers (bees removing corpses from the nest) and foragers. These two groups have completely different behavior but overlapping high titers of JH (Huang et al., 1994). Finally, although removal of the CA causes delayed foraging, bees with no circulating JH still become foragers, and only a few days later than control bees (Sullivan et al., 2000). Hence, JH and all the pathways later shown to be regulated by it do not produce behavioral specialization in a manner consistent with the idea that a given physiological state determines behavior. This is not to say that differential physiological states, and JH, are not fundamentally important, given that they clearly optimize a bee for her role in the nest, but rather that the relationship between behavior and physiology is complex.

JH and Primer Pheromones. Nursing physiology and behavior has been well explored, and it is in this context that work on the interaction between JH and primer pheromones has taken place. Pankiw et al. (1998a) showed that queen mandibular gland pheromone (QMP) treatment (extracted compounds placed into a hive) causes a delay in foraging, the opposite of the effect of JH treatment (figure 12.4). Later work suggested that QMP may directly downregulate JH and Vg (Pankiw et al., 1998a, Fischer and Grozinger, 2008). Likewise, Le Conte et al. (2001) showed in lab and field studies that brood pheromone (BP) also downregulates JH production in the presence of QMP. BP application also delays foraging (Le Conte et al., 2001).

JH and USP in the Fat Body. Ultraspiracle (*usp*) is a transcription factor (TF) that is upregulated in the fat body in response to JH in other insects, and early research confirmed the same pattern for honey bees (Hiruma et al., 1999, Jones and Sharp, 1997, Barchuk et al., 2004, Li et al., 2007, Koyama et al., 2014). We focus here on a study by Ament et al. (2012), which brought together several lines of inquiry surrounding USP, JH, and the caste-specific control of gene expression. This study showed that RNAi knockdown of USP in the fat body

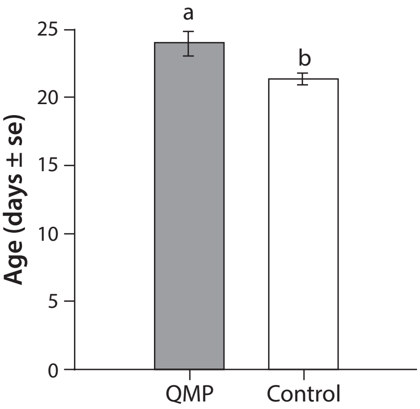


FIGURE 12.4. QMP treatment increases the age at first foraging (*from Pankiw, 1998a*).

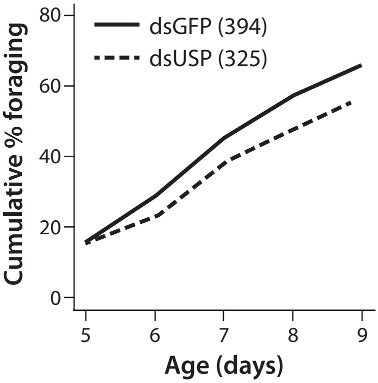


FIGURE 12.5. Knockdown of USP in the fat body delays the start of foraging (*from Ament et al., 2012*).

causes a delay in foraging (figure 12.5). They identified 85 differentially expressed genes (DEGs) that are responsive to RNAi knockout of USP in the fat body. About half of these DEGs are also differentially expressed between nurses and foragers, based on past studies. They then conducted chromatin immunoprecipitation sequencing (ChIP-Seq) to identify targets of USP. This identified 117 genes that are also DEGs between nurses and foragers. The USP cis-regulatory element was distant from the transcription start site (TSS) for most of these genes, suggesting that USP is a coregulator of these genes in conjunction with other TFs. Scans of the promoter regions of the USP-responsive genes then identified several likely candidates for these other TFs. Finally, by testing responsiveness to JH in bees with USP RNAi knockdown, it was shown that bees without functional USP are less responsive to JH, suggesting that, in general, USP mediates the effects of JH on the fat body.

This body of work led to a sophisticated model for future testing. Of chief importance, the model proposes that JH binds to Met, its receptor, which triggers formation of a protein complex initiating gene expression. Positive feedback from some of the coregulators maintains the state of high expression. In the absence of JH, the complex does not form. Ecdysteroid receptor is part of this complex, suggesting that ecdysteroids may be more important than previously thought for the control of DOL (Robinson et al., 1991). A combination of studies in model systems, tissue culture, other social insects, and honey bees will undoubtedly follow up on this impressive body of work.

BIOGENIC AMINES

Biogenic amines act as neurotransmitters, neuromodulators, and neurohormones in the nervous system (Taylor et al., 1992, Roeder, 1994). Changes in their activity are thus a potential mechanism by which the nervous system can be fine-tuned to respond to some task stimuli, while ignoring others—a core aspect of DOL. Much work has explored which of these molecules may do this, and two biogenic amines, octopamine (OA) and dopamine (DA), have shown the clearest association with the DOL system.

Which Biogenic Amines Underlie Caste-Specific Behavior? The earliest studies explored titers of DA, serotonin, tyramine, and OA in the brain between castes. This work showed that DA and OA are higher in forager than in nurse brains (Taylor et al., 1992). OA was shown to be high in the antennal lobes, although it is found elsewhere, and to show effects that are independent of age (Wagener-Hulme et al., 1999, Schulz and Robinson, 1999, 2001, Schulz et al., 2003). This was shown in studies of single-age-cohort colonies in which precocious foragers were shown to have higher titers of OA than nurses of the same age. DA, in contrast, was different between normal-age-range nurses and foragers, but did not show a difference in titer in reverted nurses (old nurses) relative to same-age foragers (Wagener-Hulme et al., 1999). Thus, its effects were interpreted as being age, but not caste, related. This may not be the best conclusion to draw, however, since reverted nurses are not identical to normal-age nurses in physiology or behavior. Hence, DA can be interpreted as being associated with normal-age nursing behavior. Later work by the Mercer lab supports this view (Beggs et al., 2007, Beggs and Mercer, 2009). Serotonin and tyramine were not shown to have consistent caste- or age-related changes in titer (Schulz and Robinson, 2001, Wagener-Hulme et al., 1999).

Later work built on the studies, showing titer differences by exploring expression patterns of the relevant receptors, while also looking for interactions between biogenic amines and core endocrinological pathways. OA receptor expression patterns, for example, also suggest that this amine is involved in generating caste-specific behavior (Reim and Scheiner, 2014). Lehman et al. (2006) further showed that the enzyme that produces OA, beta-hydroxylase, is higher in forager than in nurse brains. Kaatz et al. (1994) showed that OA treatment causes an increase in JH release from the CA. OA treatment also causes precocious foraging (Schulz and Robinson, 2001). Further, OA treatment of bees with their CA removed caused precocious foraging at the same

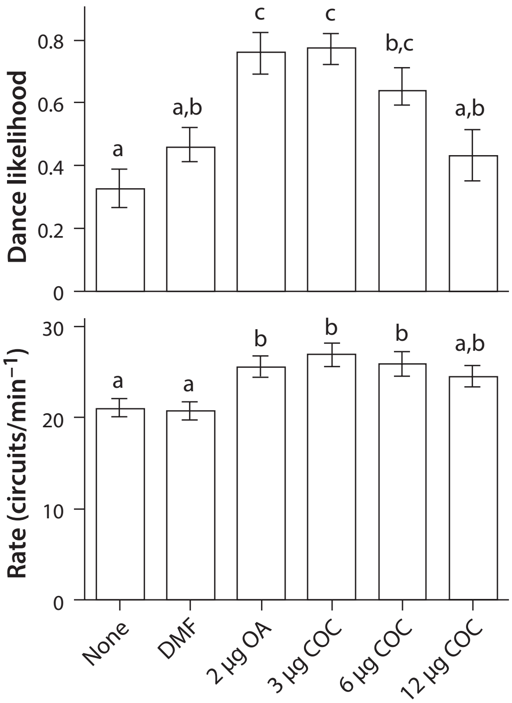


FIGURE 12.6. Application of octopamine (OA) or cocaine (COC) dissolved in DMF increased the likelihood of waggle dancing (top) and the tempo of dancing (bottom) (*from Barron et al., 2009*).

rate as controls (Schulz et al., 2002). This suggests that OA can act independently of JH, probably because it is downstream.

How Do Biogenic Amines Control Caste-Specific Task Repertoires? Foragers and OA. Work on forager behavior and biogenic amines has largely focused on how OA titer affects foraging choices or activity rates. Barron et al. (2002) showed that OA treatment (via feeding) increases responsiveness to BP. BP levels affect pollen foraging rates and, accordingly, bees treated with OA foraged more than controls. Barron and Robinson (2005) then showed that OA treatment did not affect undertaking rates, a MAB task, or queen attendance, a nursing task. In another follow-up study, Barron et al. (2009) showed that OA affects waggle dancing (figure 12.6). Treated bees danced for longer and were more likely to dance than control bees for the same feeders. In a similar study, Giray et al. (2007) found that OA-treated foragers were more likely to collect water than were sham-treated controls.

Nestmate Recognition and OA. Robinson et al. (1999) explored a role for OA in the process of nestmate recognition. Bees injected with either of two OA agonists were more likely to be aggressive to nonnestmates than to nestmates (and also in comparison to bees injected with saline). An OA antagonist blocked this effect. The authors interpret this effect as being consistent with a role for OA in priming the attention of bees to nestmate recognition cues.

Dopaminergic Signaling and Nurse Bees. Early studies by the Mercer lab looked at biogenic amine function independent of the honey bee caste system. This work led to the cloning of the receptors for DA, for example, and to the mapping of where in the bee brain various biogenic amine receptors are to be found (Mercer and Flanagan, 1988, Kokay and Mercer, 1996, 1997, Kirchhof and Mercer, 1997, Kokay et al., 1999, Kirchhof et al., 1999). The key result linking this body of work to studies of DOL was associated with tying studies of the primer pheromones controlling nurse bee behavior to dopaminergic signaling. Beggs et al. (2007) showed that bees treated with QMP have lower dopamine titers in the brain relative to control bees (figure 12.7). They then showed that the dopamine receptor, *amdop1*, decreases in the brain in response to QMP treatment. Because dopamine acts via the secondary messenger, cAMP, they then showed that QMP affects levels of this molecule in the mushroom bodies. This study also identified the component of QMP that was responsible for these effects, homovanillyl alcohol (HVA), a molecule with a strong structural similarity to DA. By repeating the QMP studies with HVA alone, it was shown that HVA produces the complete response found with QMP (figure 12.7). Finally, QMP treatment was shown to lead to lower activity levels, an effect that could be reversed with a pharmacological treatment to increase dopamine levels in the brain. Nurses have low activity levels, so this is a key link between this molecular work and behavior. Beggs and Mercer (2009) in a follow-up study expanded and clarified the known effects of HVA by showing that it causes a dose-dependent inhibitory effect on cAMP levels in neurons expressing *amdop3*. They further showed that *amdop3* is expressed in mushroom bodies. They also found that HVA does not interact with the OA receptor in the mushroom bodies.

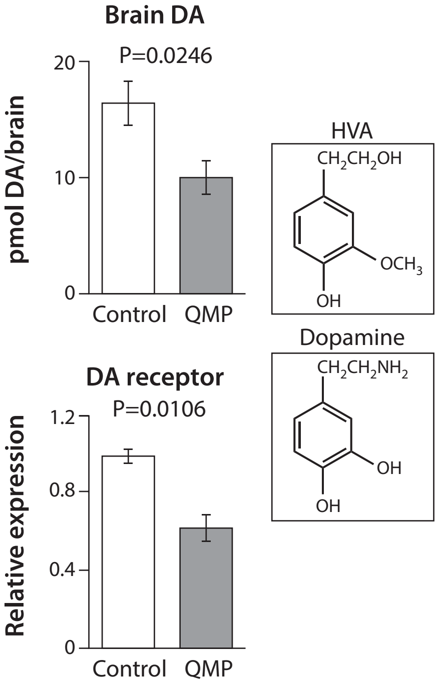


FIGURE 12.7. QMP treatment causes downregulation of brain dopamine (DA) levels and decreased expression of the DA receptor in the brain. HVA, a DA mimic, is the relevant component of QMP eliciting these effects (*from Beggs et al., 2007*).

Vergoz led studies concurrent with those of Beggs that addressed other topics associated with QMP and dopamine signaling. First, she showed that QMP disrupts aversive learning (Vergoz et al., 2007a). Control bees were shown to learn to associate an aversive stimulus (electric shock) with an odor, using the PER bioassay. Bees treated with QMP were unable to form this association. Because dopamine does not affect appetitive learning in *Drosophila*, another study tested whether QMP affects this form of learning. The ability to form an association between an odor and a reward was not affected by QMP treatment. Thus, QMP seems to selectively affect the ability of nurses to learn to avoid aversive stimuli. The authors speculated that this has to do with nurses not forming an aversive avoidance of QMP and, consequently, the queen.

A follow-up study by Vergoz et al. (2009) showed that early exposure to QMP is necessary for it to control nurse bee behavior. Specifically, only bees exposed to QMP early in life were later attracted to QMP, which is typical for nurses. They further showed that QMP treatment in young bees reduces levels of the dopamine receptor *amdop1* in the antennal lobe. *amdop3* and the OA receptor were also shown to be higher in the antennae of bees that are attracted to QMP. *Amdop3* was further shown to fall in expression with age, as does DA titer.

SINGLE GENE STUDIES

Most early studies of the genetic basis of DOL followed the reverse genetics approach. Here, work from a model system guides the research under the assumption that there is a toolkit of genes that underlies a given trait, even across vast taxonomic distances (Toth and Robinson, 2007). This work is reviewed in Smith et al. (2008). We review the key studies, as there is not space to cover them all.

A great many studies comparing gene expression in nurses and foragers for regulatory genes in the brain have been conducted. Probably the highest profile of these was work showing that the forager gene (*for*), which was shown to underlie differential locomotory activity in larval fruit flies, is differentially expressed between nurses and foragers, as seen in figure 12.8 (Osborne et al., 1997, Sokolowski, 2001, Ben-Shahar et al., 2002). The *for* gene codes for a cGMP-dependent protein kinase. Higher expression in foragers, who travel farther than nurses, was interpreted as supporting a conserved role for this gene in flies and bees. Further, treatment of bees with cGMP increased phototaxis, a forager but not within-nest bee trait (Ben-Shahar et al., 2003). Another study showed that malvolio, a gene involved in manganese transport, is higher in foragers than in nurses (Ben-Shahar et al., 2004). Treatment of young bees with manganese was shown to increase precocious foraging. Neuropeptide Y (*NPY*) is associated with insulin signaling; after differential insulin signaling was shown in the fat body (Ament et al., 2008), it was shown that aspects of *NPY* signaling differ in the brain between nurses and foragers (Ament et al., 2011). A test of causality was not conducted for this gene, unlike for the other genes reviewed.

Less work has been done outside the brain, but the fat body is the next-best-understood tissue. Insulin-like proteins function as nutrient sensors in species across the tree of life (Tatar et al., 2003, Wheeler et al., 2006). Nutrient

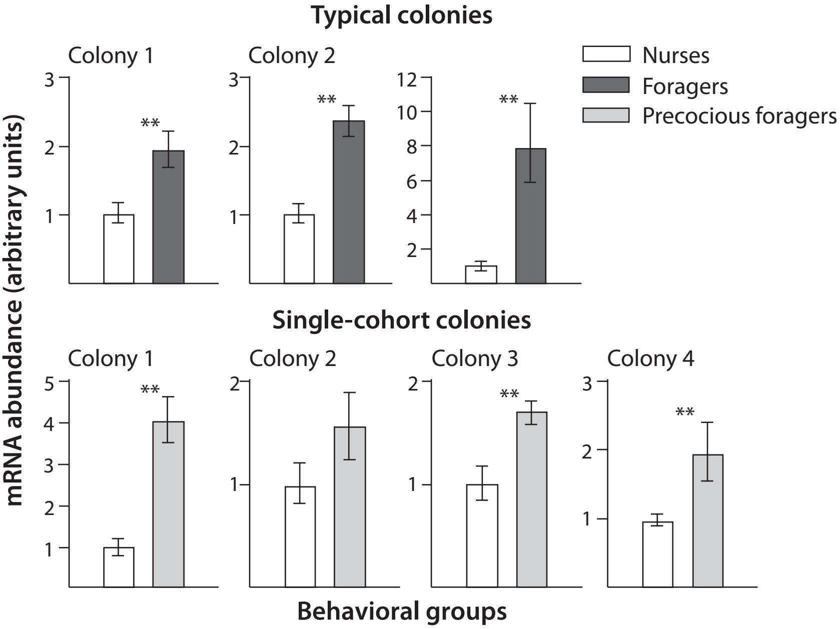


FIGURE 12.8. The foraging gene, a cGMP-dependent protein kinase, is expressed at higher levels in foragers relative to nurses (top) and in precocious foragers relative to nurses in single-cohort colonies (*from Ben-Shahar et al., 2002*).

stores strongly differ between nurses and foragers (Toth and Robinson, 2005), and aspects of insulin signaling were shown to differ in forager brains and abdomens relative to those in nurses (Ament et al., 2008). Disruption of the insulin pathway also delayed the transition to foraging, suggesting a causal link. Much work has also shown differential expression of Vg between nurse and forager fat bodies (Rutz and Luscher, 1974, Fluri et al., 1982, Amdam et al., 2003a, 2006b, Nelson et al., 2007).

Genomic Studies

The sequencing of the honey bee genome has led to a surge of studies taking a genomics approach to the genetic basis of DOL. Many of these studies use similar methods to the work just reviewed, with the exception that the entire transcriptome is explored rather than a handful of chosen genes. We break up this work by tissue.

BRAIN STUDIES

Prior to the arrival of next-gen sequencing, microarrays were the primary tool, and many thousands of DEGs were identified between the brains of nurses and foragers (Whitfield et al., 2002, 2003). Microarray studies were then conducted in which every known causative agent, from earlier work, was varied. JH and manganese treatments, for example, were used to identify genes responsive to these compounds. All this work was then compared and contrasted to produce a list of the best candidate genes for regulating DOL (Whitfield et al., 2006c). Computational studies followed this work, making further use of the extensive microarray data. One study used statistical network approaches to identify the strongest TF candidates for the control of DOL (Chandrasekaran et al., 2011), while another used scans of the promoters of the many identified DEGs to identify candidate regulatory genes (Sinha et al., 2006).

More recent work has taken a tissue-specific approach (B. R. Johnson et al., 2013). The pars intercerebralis (PI), for example, is the site of neurosecretory cells in the bee brain (Wheeler et al., 2015). In mammals, neurosecretory cells receive pheromone inputs from sensory structures and control downstream endocrine responses, making this brain region an ideal place to look for more fine-scale control than in the studies of whole brains. Many DEGs were found in the PI: thousands between nurses and foragers and hundreds in response to JH treatment. Of particular interest are USP, neuropeptide F, and several other genes previously identified in the whole-brain studies.

Another recent study that has probed deeper was conducted by Khamis et al. (2015). They used CAGEscan to experimentally link TFs to the control of genes underlying DOL. CAGE sequences the 5’ end of the mRNA transcript, which gives information about the transcription start site (TSS) and thus which TFs are involved in the gene’s regulation, since their binding motifs should be present on the transcript. They identified 26 TFs that were differentially expressed between nurses and foragers, and half of these had binding motifs associated with numerous DEGs associated with caste. A handful of TFs were predicted to regulate a large fraction of the genes upregulated in foragers. These include *nf-kb*, *pax6*, *egr*, *hairy*, and clockwork orange. The study also identified about 650 genes with variable TSSs between nurse and forager brains.

MASTER CASTE REGULATORS

Johnson and Jasper (2016) used a 10-tissue-specific RNA seq dataset to explore DOL from the toolkit perspective. They suggested that a hierarchy of regulatory genes likely controls DOL, such that some are expressed organism-wide and signal caste state, while others should be tissue specific and involved in translating the organism-wide signals into particular tissue- and caste-specific patterns of gene expression. They confirmed this by showing that most TFs are tissue specific and only a handful of candidate regulatory genes show organism-wide patterns of differential regulation between nurses and foragers (table 12.2). Two of the best candidates for master caste regulators, based on these datasets, are Kruppel homolog 1 (*krh-1*), which is associated with JH signaling (Grozinger and Robinson, 2007, Fussnecker and Grozinger, 2008, Minakuchi et al., 2008, Shpigler et al., 2010), and double sex (*dsx*), the sex determination gene (Beye, 2004, T. M. Williams et al., 2008, Tanaka et al., 2011, Kijimoto et al., 2012). *Dsx,* in particular, since the publication of Johnson and Jasper (2016), has attracted some attention in studies of DOL in bees and other social insects (Klein et al., 2016, Velasque et al., 2018, Jia et al., 2018). The idea being tested, proposed in Johnson and Jasper (2016), is that *dsx* signals sex status to a tissue with its sequence (it has alternative splicing between males and females), but differential expression of this gene could then be used to signal caste or other socially relevant status.

Genomic Responses to Pheromones

Primer pheromones regulate the development and physiology of the receiver and are thus highly relevant to the study of the genetic control of DOL. Two pheromones in particular, queen mandibular gland pheromone (QMP) and brood pheromone (BP), are central actors and have been the subject of study. They are not the only such pheromones, however. The pheromones produced by the Dufour's gland in queens, and likely other glands as well, also probably have primer effects and should receive more attention.

Grozinger et al. (2003) conducted cage experiments in which bees were treated with QMP. Hundreds of genes changed transiently in expression in response to QMP treatment and 19 genes showed consistent changes. *Kr-h1*, a JH-responsive TF, was one of these (Fussnecker and Grozinger, 2008, Shpigler et al., 2010). A comparison of the genes that responded to QMP treatment

Table 12.2. Regulatory genes with broad patterns of differential expression across nurses and foragers

Entrez ID	Name	Ganglion	HPG	Malpighian tubules	Mandibular gland	Midgut	Muscle	Nasonov gland	Sting gland
100576395	Kruppel homolog 1	Forager	Forager	Forager	Forager	Forager	Forager	Forager	Forager
726204	hairy	Forager		Forager	Forager	Forager	Forager	Forager	Forager
102655703	zinger finger 250	Forager	Forager	Forager	Forager	Forager		Forager	Forager
409822	E(spl)-like protein		Forager	Nurse	Forager	Nurse		Forager	Forager
724465	enhancer of split		Forager	Nurse	Forager		Forager	Forager	Forager
725126	double sex	Nurse	Forager	Forager	Forager	Forager		Forager	
410309	ecdysone-induced 75		Forager					Forager	
410326	Kruppel-like factor 10		Forager	Forager				Forager	
410468	hairy	Forager	Forager	Forager				Forager	
411207	slow border cells		Forager	Forager				Forager	
411809	enolase-phosphatase E1		Nurse	Nurse	Nurse			Nurse	Nurse
412770	TF E2F2-like		Nurse		Nurse			Nurse	Nurse
725263	tumor p53-inducible nuc 2		Forager	Forager	Forager	Forager		Forager	
725614	cycle		Forager	Forager	Forager			Forager	Forager
727091	foxo		Forager		Forager		Nurse	Forager	Forager
406066	juvenile hormone esterase	Nurse		Nurse	Nurse	Nurse		Nurse	
409241	prohomone 4			Forager	Nurse	Forager	Forager	Forager	

Note: “Nurse” or “forager” refers to the caste in which the gene is upregulated.

and those that were differentially expressed between nurses and foragers showed that QMP tends to regulate those genes with higher expression in nurses. This is consistent with QMP being a core regulator of nursing physiology and behavior.

With respect to BP, Alaux et al. (2009b) conducted a study using a triple-cohort colony (colonies with three age cohorts designed to mimic a natural colony's caste system), showing that exposure to BP downregulates genes typical of forager expression patterns. Further, BP was shown to discourage foraging in young bees, while encouraging foraging in older bees.

Proteomic Studies

Transcriptomic studies have dominated the genomic approach to honey bee DOL. This is because the relevant methods are simpler and the results are easier to interpret. Nevertheless, a full understanding of the mechanistic basis of any trait must include both transcriptomic and proteomic studies (Chan et al., 2011). There is even the argument that the proteome is the more central aspect of the problem, as proteins do most of the actual work, not their RNA precursors.

Hernandez et al. (2012) conducted a study of the proteome of nurse and forager brains, identifying 2742 proteins, 17% of which differed between the castes. Han et al. (2015) conducted a proteomic study focused on neuropeptides and identified 158 neuropeptides stemming from 24 precursors. Tachykinins were quite diverse with 24 neuropeptides. They also found variable numbers of neuropeptides produced by different ages (castes), having collected bees at emergence, 7 days, 14 days (nurses, not MABs), and foragers. In another study from the same lab, they used a proteomics approach to identify all transmembrane proteins as well as sites of phosphorylation. Phosphorylation is a form of posttranslational modification that affects protein function, typically by activating or deactivating the protein or changing its interaction partners. They identified over a thousand transmembrane proteins, 417 of which were found to have phosphorylation sites. Most of the proteins were phosphorylated by kinases in the MAPK, CDK, and CK2 families. They also found small numbers of proteins uniquely expressed by each age group.

13

Communication, Labor Allocation, and Collective Decision Making

Labor allocation (also commonly called task allocation) is the study of how colonies allocate the optimal number of workers to tasks that change in demand through time and space (Traniello and Rosengaus, 1997, Anderson and Ratnieks, 1999, Beshers and Fewell, 2001). In this chapter, we cover the ideas that underlie the study of task allocation and give honey bee examples. For some topics, we both review the data and present a critical analysis. This is necessary because some otherwise useful ideas have gaps in their supporting data. It is hoped that a critical review will trigger deeper research into these topics.

Signals and Cues

A core topic in task allocation is how workers acquire the information necessary to determine what task to perform (Seeley, 1998, Jeanne, 1996, Beshers and Fewell, 2001, Johnson, 2010b). The roles of cues and signals have been central to our understanding of this question (von Frisch, 1967, Free, 1987). A cue, as traditionally defined, is a source of information that has not been shaped by natural selection to provide information (Lloyd, 1971, Bradbury and Vehrencamp, 1998, Seeley, 1998). The tracks left behind by a deer are cues. These sources of information are an unavoidable consequence of the laws of physics. In general, cues can be perceived with any sensory modality. Important cues for bees are encounter rates, floral odors on the bodies of returning foragers, wait times, and so forth (Seeley, 1998). Signals, in contrast, are sources of information that have been shaped by selection to convey information. Signals evolve from cues such that both the information content is increased and the sensory equipment, and cognitive mechanisms, used to receive and process the information are elaborated (Bradbury and Vehrencamp, 1998). Once received, signals change the behavior of the receiver.

Signals and cues work hand in glove in labor allocation, but in this section we discuss each separately. We reviewed in chapter 11 the simple feedback loop that controls the balancing of effort between nectar foragers and processors. When a forager returns to the nest to unload her nectar, there are many cues available to her to infer the balance between the two activities. These include the number of potential receivers encountered, the time to complete unloading, how far into the nest the bee must travel to find a receiver, the total distance traveled searching for receivers, the number of rejections, and so forth. Although cues clearly play a major role here, the exact cue the foragers use in this situation is difficult to identify. This is because many cues provide the same information. For example, variation in both the time until unloading and the distance of the search are probably correlated, and there is no guarantee that the one with the strongest correlation is the one used. This example illustrates a problem that is generally true in this field. It would likely require a neuroethology approach, not a behavioral ecology approach, to identify the cue (or cues) used in any given situation; given the difficulty of such studies in freely living bees, this has yet to occur.

Signals, in contrast to cues, are sometimes easier to study. A commonsense approach involves examining the context in which the signal is sent along with recording the response of the receiver. This often allows one to decipher a signal's message and meaning. For example, stop signals are mechanical/acoustic signals often produced by foragers after they have been harassed while feeding at a feeder, and presumably at flowers (Nieh, 1993, 2010). The response of waggle dancers, who are the receivers of this signal, is to stop dancing. Hence, the context is one of danger and the response is to stop sending bees to that place. This straightforward approach has been used to solve, in part, the meaning of several honey bee signals. Our discussion of the tremble dance in chapter 11 is another example. Bees were observed to produce the signal after having difficulty unloading their nectar, and bees who received the signal were observed to increase their rate of nectar receiving (Seeley, 1992b).

Before reviewing the known signals and their functions, we must make a few more general points about honey bee signaling. First, many signals probably have multiple meanings both for bees in the same caste and particularly for bees in different castes. A signal such as the queen pheromone signals

Table 13.1. Honey bee mechanical signals

Signal	Class	Known function
Waggle dance	Discrete	Recruitment
Tremble dance	Modulatory?	Regulates nectar processing
Shaking signal	Modulatory?	Regulates worker activity level
Stop signal	Discrete	Stops waggle dancer
Worker piping	Modulatory?	Warms up swarm
Queen piping	?	Signals queen presence
Buzz run	Discrete	Signals swarm departure
Nest defense buzz run	Discrete	Signals ownership of nest site
Swarm streaking	Discrete	Guides swarm to new location
Grooming invitation	Discrete	Requests grooming
Antennation	Discrete + modulatory?	Begs for food

queen presence (and suppresses ovary development) to workers, while it is a mating signal for drones (Le Conte and Hefetz, 2008). This is well known for chemical signals, but there is no reason not to suspect that it is true for mechanical and acoustic signals as well. Context defines the meaning of the signal. Second, many signals are used in more than one context. This could mean that they have more than one meaning, or it could mean they elicit a response that is useful in many different situations. The shaking signal, for example, is thought to vary activity rate, which can be useful in many contexts (Seeley et al., 1998, Nieh, 1998, Schneider and Lewis, 2004).

Table 13.1 lists the known nonchemical signals used by honey bees. Chapter 14 is focused on chemical communication, so chemical signals are discussed there. The nonchemical signals are mainly mechanical and acoustic, as cavity nesting bees do not use visual signals in the darkness of the nest (although they may when swarming). We now review what is known of the message and meaning of each signal. We spend little time on descriptions, because they are less effective than simply informing the reader that videos of all of these signals are available on YouTube. Watching a tremble dance is more informative than any verbal description accompanied with a line drawing.

Signal Glossary

Waggle dance: It would not be an exaggeration to say that an entire book could be written about this signal alone. Here we give a succinct description of the signal and its adaptive function. The waggle dance is a stereotyped, repeating figure-eight pattern produced by a returning forager (figure 13.1). There is both a waggling phase (when the abdomen is going from side to side) and a return phase to the dance. The waggling phase is also sometimes called the run phase of the signal (von Frisch, 1967). Bees produces airborne sound during the waggling phase at about 250 Hz in short pulses (Esch et al., 1965, Esch and Wilson, 1967, Michelsen et al., 1986, 1987, 1992, Kirchner et al., 1988). Wing movements during the run phase also produce a narrow jet, or broad flow, of air behind the dancer, depending on the position of the wings (Michelsen, 2012). The narrow jet of air is thought to be used by the follower to orient in the direction of the dancer (Michelsen et al., 1987, 1992, Michelsen, 1993, 2003). The broad air flow may aid in the spread of pheromones, which are also emitted during the dance (Thom et al., 2007, Gilley et al., 2012, 2018, Gilley, 2014). The sound, pheromones, and substrate-borne vibrations together alert bees near the dancer that waggle dances are being produced, while also helping the receivers localize the dancer in the dark hive.

With respect to function, the waggle dance is used by foragers to tell other foragers, called recruits, where something of value is located. The signal (the waggling phase) contains information about the distance and the direction to the location. The length of the waggle run part of the dance indicates distance, while the angle relative to vertical (against gravity) indicates the direction with reference to the azimuth of the sun (figure 13.1). In the case of dances for food, the odor of the flower on the dancer's body (a cue, or perhaps a signal from the flower) is also important. A recruit must typically follow about five dances to extract the necessary information, and multiple trips are usually necessary to find the advertised location (Judd, 1995, Seeley, 1995). Karl von Frisch, one of the founders of the field of animal behavior, is the person who deciphered the meaning of this signal, along with many collaborators. He was awarded the Nobel Prize mainly for this work, although he made other important discoveries as well, which we have covered in other chapters. The

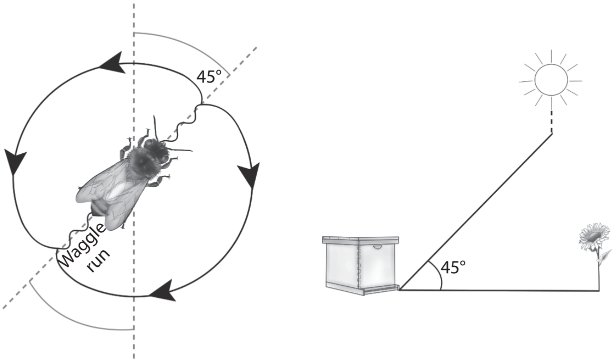


FIGURE 13.1. The waggle dance indicates the distance and direction to a location such as a food source or nest site. The length of the waggle run (indicated by the wavy line) encodes distance, while the angle relative to vertical is used to inform direction relative to the azimuth of the sun (*redrawn based on Barron and Plath, 2017*).

interested reader is referred to von Frisch (1967), *The Dance Language and Orientation of Bees*, a wonderful book reviewing decades of research.

The round dance, once thought to be a distinct signal, is now known to be a waggle dance with a short waggle run. First, bees produce sound during the brief run phase that indicates direction (Kirchner et al., 1988). Second, there is no meaningful switch in the functional character of the signal as the distance indicated goes from near the hive (5–10 m) to far (Gardner et al., 2008). Finally, recruits were shown to use the directional information (controlling for olfactory signals) for sites as close a 10–20 m to the nest (Griffin et al., 2012). It is the case, however, that there is considerably more scatter in the directions signaled for near versus far sites.

Tremble dance: The tremble dance was first noticed by von Frisch. In some sense, it looks vaguely like a waggle dance but with no order. The bee shakes erratically in all directions as she walks about the nest. The function of this signal was not known until Seeley (1989b) conducted a study showing that, when nectar receivers are removed, the nectar foragers produce large bursts of this signal throughout the hive (von Frisch, 1923, Seeley, 1992b, Kirchner and Lindauer, 1994, Seeley et al., 1996). This quickly leads to a higher rate of nectar receiving in the hive. A function, therefore, is to attract MABs to nectar processing. Seeley proposed that too long a wait time while attempting to unload nectar triggers foragers to produce this signal. Another study showed, however, that many bees produce tremble dances immediately upon entering the hive, calling this result into question (Thom, 2003). A simple explanation for the discrepancy might be that the tremble dance is used in two related contexts. First, it serves as an indicator to the MABs of foraging effort. If a constant percentage of foragers produce the signal as they work (immediately upon entering the hive, for example), then this would signal their current activity to the MABs, thus ensuring a maintenance of the current level of nectar receiving. This would explain the work of Thom. Second, when a strong mismatch occurs between foraging and receiving, typically due to the start of a nectar flow, then the foragers can rapidly produce more tremble dances to attract more MABs to nectar processing. This would explain Seeley's result.

Shaking signal: The shaking signal, also called the dorsoventral abdominal vibration signal (DVAV), was initially described almost a hundred years ago (Haydak, 1929). This signal involves a bee grabbing another bee and apparently shaking her. Bees will also shake the comb. The shaking—a vibration, really—is at a frequency of about 16 Hz and lasts for about a second (Gahl, 1975). It is a common signal, and bees can be seen performing it in an observation hive at most times. There is an overall pattern to its production, however. A peak in signals is produced each morning just after the commencement of foraging, and a second smaller peak occurs late in the day toward the end of foraging (Schneider et al., 1986a, 1986b, Nieh, 1998). Consistent with this, observations of foragers show that when they find high-quality food sites first thing in the morning, their first couple of returns to the hive are not spent waggle dancing, but rather producing the shaking signal (reviewed in Seeley, 1995). The signal is thus akin to a work bell (Schneider, 1987, Nieh, 1998). It may signal that an increase in work rate is needed at the start of the day and that many activities are ending as the sun goes down.

Grooming invitation: This is a simple signal that allows a bee to request grooming from another bee (Land and Seeley, 2004). It seems to be a classic case of signal evolution by exaggeration of a cue (Bradbury and Vehrencamp, 1998). When bees are grooming, they make characteristic movements as they use their legs to reach difficult places. The grooming signal is an exaggeration of these movements without the actual grooming. This is a difficult signal to verbally describe, so the interested reader is encouraged to search for a video of it on YouTube. When bees perform this signal, another bee quickly responds by intensely grooming the sender.

Worker piping: Worker piping was first discovered about 100 years ago by Armbruster but was not described until the 1960s (Armbruster, 1922, Esch, 1967). This is an acoustic, or substrate vibration, signal. The bee produces a burst of sound at 350 Hz with her flight muscles as she presses her thorax against the comb (Pratt et al., 1996). This signal is used in many contexts, the function of which is known in only a few cases. As part of the initial phase of swarming, workers produce a burst of piping signals immediately preceding swarm departure (Rangel and Seeley, 2008, Rangel et al., 2010b). During house hunting, the scouts use this signal to tell the nonscouts, who are in a resting state, to warm up and prepare for takeoff (Seeley and Tautz, 2001). In both of these cases, the signal coordinates mass departure. Piping is also used in the context of nectar foraging (Pratt et al., 1996, Thom et al., 2003), although its role is unclear. Thom et al. (2003) showed that piping signals are mainly produced by tremble dancers. It might be that these studies by Pratt and Thom confuse the piping signal with the stop signal (covered next). Piping by laying workers in queenless colonies has also been observed (Ohtani and Kamada, 1980).

Stop signal: The stop signal was initially identified by Esch (1967), who thought it was a begging signal. It may be the same signal as worker piping, but as this is not clear we cover it separately. This signal involves a short burst of sound plus a head-butting movement against another bee. This signal is targeted to waggle dancers, and their response to its reception is now known to involve the cessation of dancing (Nieh, 1993, Pastor and Seeley, 2005). Nieh (2010) later showed a higher level of sophistication in the use of this signal than had been previously suspected. Foragers who are harassed at feeders produce many of these signals, which they direct at waggle dancers advertising the location at which the harassment occurred. They do this by targeting bees with the same odor as the site they visited. This signal thus seems to be involved in shutting down recruitment and foraging to a particular site or type of plant (Johnson and Nieh, 2010). The signal is also used in the house hunting process (Seeley et al., 2012).

Queen piping (or tooting): Queen piping was one of the first signals discovered (Butler, 1609, Huber, 1792). This is likely because it is one of the few signals that can be heard with the naked ear without effort (Armbruster, 1922, Hannsson, 1945, Simpson, 1964). As for worker piping, this is a substrate-borne vibration, so the queen presses her thorax to the comb and produces short bursts of sound at about 400 Hz (Michelsen et al., 1986). We discussed in chapter 8 the function of this signal during swarming, as the virgin queen pipes frequenting both in and out of the queen cup. This seems to signal her presence to the workers, although it may also modulate worker aggression, as workers who perceive the queen piping signal freeze (Simpson and Cherry, 1969, Spangler, 1971, Fletcher, 1978, Kirchner, 1993a). Playing artificial queen piping signals to small colonies induced them to swarm (Simpson and Greenwood, 1974).

Buzz run: A burst of these signals precedes swarm departure both from the home nest and from a temporary site after a decision has been made on a new home (Rangel and Seeley, 2008). In the context of lift departure to a new home, the sender burrows quickly through the cluster of bees, producing buzzing sounds (Rittschof and Seeley, 2008).

Nest defense buzz run: Nest scouts guarding a potential nest produce this signal (Rangel et al., 2010a). The signal itself involves a bee running about in a zigzagging fashion while buzzing and occasionally head butting the receiver of the signal. This apparently signals to bees from other colonies that the cavity is taken.

Swarm streaking: The overwhelming majority of the bees in a flying swarm have no idea where they are going, yet the swarm flies efficiently to its destination. This is achieved by scouts signaling the flight direction with rapid fights through the swarm in the direction of travel (Lindauer, 1955, Seeley et al., 1979, Schultz et al., 2008). This is a striking signal to observe, as the scout bees are flying probably as fast as they are physiologically capable, which is quite impressive.

Basic Concepts in Collective Behavior

Modulatory Signaling

Although we know a great deal about honey bee signaling, we still do not completely understand how signals coordinate the activities of bees in different castes. The sender-receiver approach to signal decipherment we discussed previously has been the workhorse of this field, but it fails in many cases. The most important cases relate to what are known as modulatory signals (Hölldobler and Wilson, 1990). Bees receive hundreds, or even thousands, of these signals per day and their accumulation modulates behavior. To refer to our review of signals, the shaking signal, the tremble dance, and worker piping are modulatory signals (Nieh, 1998). These signals are not sent from one bee to another but are broadcast by the sender to hundreds of bees across the nest. This broadcasting can take up to an hour, though 10–20 minutes is more typical (reviewed in Seeley, 1995).

Understanding the meaning of a modulatory signal that is received at a variable rate, and that has a modest quantitative effect on the receiver, is much harder than understanding something like the waggle dance, which is discrete and sends a packet of information that is immediately used. There is some evidence that activity changes after reception of a shaking signal, for example, but it is weak (Nieh, 1998). The same can be said for part of the tremble dance story. Nectar receiving increases after the signal is broadcast, providing a group-level function for the signal. But what the signal means to an individual bee who encounters a tremble dancer is not clear. In other words, how the tremble dance causes the increases in nectar receiving is unclear. To an individual receiving the signal, it could directly mean change task to nectar receiving; or it could mean something indirect that accomplishes the same goal, such as go to the dance floor, increase overall activity, quit your current task and look for a new one, or conduct a patrol (change location in the nest) (Johnson, 2009b). Likewise, worker piping could mean stop your current task, freeze for a moment, look for a new task, and so forth. There are many behaviors that could have the same group-level effect, meaning that showing a group-level effect is not enough to decipher the individual-level meaning of a modulatory signal.

To decipher the meaning of modulatory signals requires automated tracking of thousands of bees in a nest of reasonable size in various contexts. This would allow one to statistically infer the minute changes in behavior that accrue with each reception of a modulatory signal. Experiments such as Seeley et al. (1996), for example, in which the nectar influx was radically increased from one day to the next, could be repeated with automated tracking of tremble dance recipients. The silver lining here is that automated tracking is almost of sufficient quality for this to happen (Blut et al., 2017, Gernat et al., 2018). Importantly, the researchers in this field will need to use full-size observation hives (at least two frames) in order to study these signals (plate 13), as they (and most other honey bee behaviors) are difficult, if not impossible, to meaningfully study in small groups of bees. Hopefully, within the next decade or so we will acquire a finer-grained understanding of how honey bee colonies coordinate their activity via the many modulatory signals continuously being sent and received by nearly all bees (Wilson and Hölldobler, 1988).

Specialization

Numerous studies suggest that bees may specialize in particular tasks (Visscher, 1983, Moore et al., 1987, Breed et al., 1992a). Specialization is here defined as a bias in the performance of some task relative to the background performance rate of that task. A bee that spends 10% of her time guarding the entrance, for example, would be a specialist if the average rate of guarding is 3%. This kind of specialization was shown for guarding, undertaking, allogrooming, and wax working in the middle-age caste (Visscher, 1983, Kolmes, 1989, Trumbo et al., 1997, Johnson, 2002). In foragers, some bees are more likely to collect pollen rather than nectar and vice versa (Page et al., 2006, Siegel et al., 2012, Ihle et al., 2015b). In all these cases, specialization is far from complete, and all bees seem willing to conduct any task within their caste-specific task repertoire (Seeley and Towne, 1992, Johnson, 2009b). Further, most of the work for these tasks is performed by generalist workers, as the specialists are few in number (Johnson, 2002, Perez and Johnson, 2019). This can be illustrated with data on guard bees, perhaps the most well-characterized specialists. Table 13.2 shows the number of days that bees from seven colonies were seen guarding. Most guarding is done by bees who only do the task for a brief period of time before going back to working on other tasks within the MAB task repertoire (Trumbo et al., 1997). This pattern holds for all the known cases of specialization, with the exception of allogrooming (Kolmes, 1989, Trumbo et al., 1997). Finally, specialization effects have been shown to have at least a partially genetic basis. In a colony with multiple patrilines, for example, specialists are more likely to come from some patrilines than others (Chapman et al., 2007, Duong and Schneider, 2008, Mattila and Seeley, 2011, Ernst et al., 2017).

Stimulus Response Thresholds

The stimulus response threshold concept goes back to the early ethologists (Bradbury and Vehrencamp, 1998). The idea is that there is a stimulus in the environment associated with some activity that is perceived by an animal, and

Table 13.2. Persistence of guarding behavior in middle-age bees

	Days seen guarding						
Colony	0	1	2	3	4	5	6
1	1045	99	2	12	0	2	1
2	807	99	14	4	1	1	0
3	948	103	25	10	2	2	1
4	916	88	9	5	0	0	0
5	921	36	3	0	0	0	0
6	914	68	14	7	0	0	1
7	956	85	25	10	2	2	1

Source: Moore et al., 1987.

a stimulus level above a threshold elicits a response. According to the social insect version, workers vary in their response threshold, due to genetic or other effects (often learning) such that those with the lowest threshold are the most likely to perform a task (Page et al., 1998, Bonabeau et al., 1998, Theraulaz et al., 1998, Fewell and Page, 2000, Beshers and Fewell, 2001, Pankiw et al., 2001, Weidenmuller, 2004). Bees with the lowest threshold are thought to eliminate the stimulus for their task, meaning other bees might never engage in it. This is a potential explanation for the specialization effects found in empirical studies. There is evidence both for and against this notion.

In general, it is clear that genotype affects task choice, and probably response thresholds. Sucrose responsiveness, measured early in life, predicts foraging choices (to a modest extent) later in life, for example (Page et al., 1998, Pankiw et al., 2001). How much of task allocation can be explained with a simple model, like the response threshold idea, is the crux of the matter. For example, Jones et al. (2004) explored patriline effects on thermoregulation, a task for which it is easy to manipulate demand by increasing temperature. Although patriline affected a bee's probability of fanning at each temperature, the overall patriline diversity did not go up with temperature (figure 13.2). This result was confirmed in theoretical modeling (Graham et al., 2006). Robinson and Page (1995) in an earlier study found something similar in that patriline diversity of bees engaged in undertaking (removal of corpses) did not increase under high versus low undertaking task demand.

Overall, there are many mechanisms that would blur a simple relationship between fixed genetic variation and task choice (Graham et al., 2006). Most

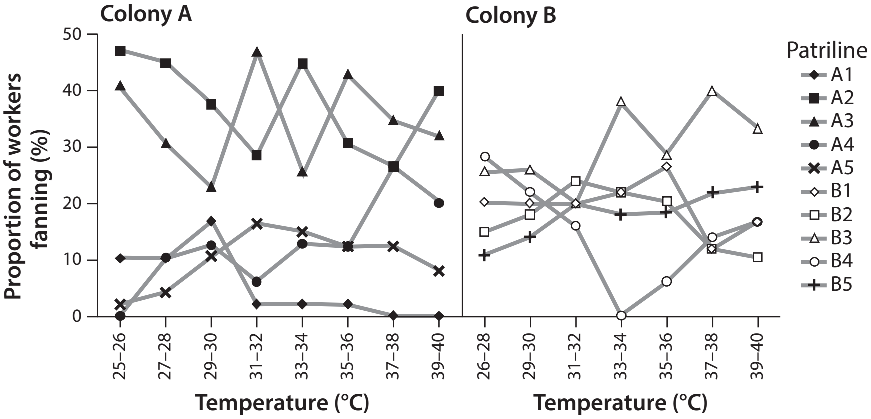


FIGURE 13.2. Proportion of bees in two colonies in different patrilines fanning in response to increasing temperature (*from Jones et al., 2004*).

importantly, bees get allocated to tasks according to colony needs by group-level feedback loops involving signals that likely alter response thresholds (Seeley, 1995, Alaux et al., 2009a). We return to this question in chapter 15 on foraging. In general, the response threshold idea is a good building block that must be part of a more complex process controlling labor allocation, but it is unlikely to be more than a partial explanation.

Genetic Variation and Task Allocation

Considerable work has explored the idea that genetic variability, which is large in honey bees due to extreme polyandry, evolved because it confers more efficient patterns of labor allocation (Page et al., 1989, Cole and Wiernasz, 1999, Jones et al., 2004, Mattila and Seeley, 2007). We first review evidence that supports this view, then some that does not. We conclude with a discussion of how this idea could be better tested.

We already briefly discussed the work of Jones et al. (2004). This study showed that open-mated colonies, which have 12 patrilines on average, have slightly less variability in broodnest temperatures than do single-patriline colonies. Work following up on this has been largely conducted by Mattila, Seeley, and colleagues. Their first study showed that single-patriline colonies grow slower and have generally lower fitness after colony founding than colonies mated 15 times (Mattila and Seeley, 2007). It was then shown that multiple-patriline colonies conduct more waggle dances and are better able to use the information in them during foraging than are single-patriline colonies (Mattila et al., 2008, Girard et al., 2011). They also showed that scouting is better conducted in multiple- rather than single-patriline colonies (Mattila and Seeley, 2011).

In contrast to these results, Page et al. (1995) compared single-patriline and four-patriline colonies and found few differences in various fitness-affecting traits. They also found great variability within the single-patriline colonies such that some showed skewed labor allocation but others did not. They suggested that the benefits found for multiply inseminated colonies may stem from the averaging effect found from mixing lots of patrilines. A modeling study found a similar effect (Myerscough and Oldroyd, 2004). Hence, for any task, the colony will have a response threshold distribution at the group level that is normal with good variation if it is composed of many patrilines. Any given patriline may be average or extreme, however, leading to variable single-patriline colony results.

Neither Page et al. (1995) nor Myerscough and Oldroyd (2004) made this point, but what their works suggests is that a relaxed selection explanation may exist for the data, suggesting that genetic diversity improves task allocation. In short, if selection for extreme polyandry is due to some factor, such as disease resistance, then task allocation will come to operate within this genetic context. Because mixing many patrilines leads to a normal distribution with a wide variance at the colony level, there would be relaxed selection, allowing single-patriline response thresholds to evolve extreme mean values that are dysfunctional in isolation. Such effects would not happen in singly mated species (the overwhelming majority of social insects), which is why their task allocation processes do not apparently suffer the problems found in single-patriline honey bee colonies.

In summary, there is support both for and against the notion that bees mate multiply to improve task allocation. This is doubly true when data from other social insects are included (Bonabeau et al., 1998, Fewell and Page, 1999, Weidenmuller, 2004, Jeanson et al., 2007, Holbrook et al., 2013). With respect to bees, although there are more studies in favor, close inspection shows that the same experimental design has been repeated many times with different behaviors. These studies also compare one patriline against many but claim to provide an explanation for extreme polyandry. To show that, however, would require testing mating levels that vary from 1 to perhaps 20 in increments. Then we could see at what number the benefit levels off. If there is no difference, for example, in task allocation between a colony with 6 patrilines versus

12, then improved task allocation cannot be an explanation for extreme polyandry (Gove et al., 2009). All in all, it is unclear what the answer is here, but future work should test the relaxed selection idea and explore a dose-dependent response in labor allocation to increased mating frequency. It would also be useful to artificially add patrilines to singly mated species with large colony sizes to see if this improves labor allocation.

Self-Organization

Self-organization (SO) is a concept that explores how order grows out of local interactions, often involving feedback loops that amplify in some manner (Turing, 1952, Deneubourg et al., 1990, Camazine, 1991, Bonabeau et al., 1996). In these situations, the pattern, or organization, is said to be the result of a decentralized process. Distributed intelligence is a synonymous term, and collective behavior, which is probably not a synonymous term, is sometimes also used to refer to the same processes. SO quickly became a prominent idea in social insect biology in the 1990s (Deneubourg et al., 1990, Camazine, 1991, Bonabeau et al., 1996, Dorigo et al., 2000, Gautrais et al., 2002). After a burst of activity on this topic, lasting about a decade, papers explicitly about SO have now fallen in number. However, the ideas are still central to our understanding of many aspects of social insect biology. Here, we first give some examples of SO in honey bee colonies to highlight the utility of these ideas. We then clear up some problems caused by exaggeration of the simplicity of social insect workers.

HONEY BEE SELF-ORGANIZATION EXAMPLES

Scott Camazine conducted several early SO studies to illustrate the utility of the idea for the honey bee (Camazine et al., 1990, Camazine and Sneyd, 1991, Camazine, 1991). Probably the most important for honey bee biology are the models of collective foraging. However, because we cover foraging in great detail in chapter 15, we now focus on two simpler SO models. The first illustrates the basic idea, and the second highlights some understudied aspects of labor allocation.

Honey bee comb tends to exhibit a characteristic pattern on its surface. The brood is in the center, the pollen is in a band around it, and the honey is on the outside. Camazine showed that a few simple rules underlying an SO algorithm can account for this pattern (Camazine et al., 1990, Camazine, 1991). First, the queen avoids the edges of the comb and prefers to lay eggs near one another, causing the brood to cluster. The workers unload pollen and nectar at random, but because nurses conduct a random search for food near the brood, they tend to empty cells near the brood first. Then, because pollen has a faster turnover than honey, at equilibrium it tends to end up in a band next to the brood where the only open cells are located. Thus, the workers do not need to have a blueprint of what the pattern is supposed to look like in order for their behavior to create it. Subsequent work suggests that this case of pattern formation may not be so simple in that there are probably more rules at play (some of which are not self-organizing), but the basic idea does not change (Johnson, 2009a, Montovan et al., 2013). No worker need have reference to the global pattern, and simple rules can underlie adaptive group-level behavior.

A second case of SO relates to optimal task allocation in MABs (Johnson, 2009b). MABs have a large task repertoire. They process nectar at the top of the nest, for example, and guard and receive nectar from foragers at the bottom. The focal question is, How do they allocate themselves to tasks that vary in demand over such a large area? First, it was observed that MABs frequently quit the task they are working on with no obvious external cause (Johnson, 2008b). They also conduct frequent patrols—long walks through the nest during which they inspect cells but do not work (Lindauer, 1952, Johnson, 2008a). Johnson (2009b) explored an SO algorithm for task allocation that makes use of these two factors. Bees can be in one of three states: working, patrolling, or inactive. When working, they search for a task and work on the first task they find. When patrolling, they conduct a long walk of variable length through the nest. Simulations showed that the searching-for-work component of the model localizes workers to tasks, while patrolling causes diffusion away from areas of high task demand. Its effect is to allow the bees, at the collective level, to check whether conditions have changed elsewhere. Thus, splitting time between working and patrolling allows for both allocation to tasks and flexibility. As in the pattern formation model, no bee has an awareness of the global pattern of labor allocation.

We have covered this second model in detail because it illustrates the importance of some factors left out of the classic response threshold model, which focuses on why workers respond to stimuli. What causes a worker to quit is just as important as what causes them to start work. Workers may work for short or long bouts, for example, and they may quit based on external conditions or an internal mechanism. Most importantly, how workers encounter the stimulus in the complex environment they live in is often the critical question in labor allocation. The fact that bees vary in their responses to stimuli is important, but just as important is which bees encounter the stimulus and how often. How much individuals move about the nest and why must therefore be considered when exploring any case of labor allocation, as it determines the rate at which workers encounter task-related stimuli.

GLOBAL INFORMATION PROCESSING

When a powerful new idea takes hold, there is always the risk that its proponents will extend it too far. This is what has occurred with SO and social insect behavior. There are now two standard statements in studies of labor allocation that stem from the SO literature. These are (1) workers have simple brains and (2) workers make use of local information. The idea is that because their brains are small, they cannot process much information and instead only pay attention to local information. Simple responses to local information then amplify into global patterns without the need for global information sharing or processing. This sounds enchanting to many, but it is a serious exaggeration, particularly for honey bees.

The common notion that insects have simple brains is based on a comparison to more complex brains. Our brains have billions of neurons, while the bee brain has only a million. The astronomical complexity of human brains, however, simply has nothing to do with whether a brain of a million neurons is simple in an absolute sense. Models of brain function (called neural network models) typically have dozens to maybe hundreds of neurons, and even these models are poorly understood as their behavior can be astonishingly complex (Yuste, 2015). A brain of a million neurons has billions of connections. In short, we do not understand the bee brain well enough to know what they can or cannot process from a neuroethological perspective, and statements to the effect of “the bee’s brain is simple” are misguided.

This brings us to the second belief—that bees only respond to local information. This is incorrect, as many studies have shown that honey bees go to great lengths to share global information. An early study by Seeley (1979), for example, tackled this question with respect to queen pheromone. The queen is a single bee, so how can she signal to the whole colony her presence, which is critical to the colony’s reproductive status? It turns out that some nurse bees act as dedicated messengers. They antennate and lick the queen to obtain some of her pheromones, and then spend 30 minutes or more scurrying about the nest sharing it with all colony members. Everyone in the colony has access to this global information. Seeley's work on the tremble dance, which should be familiar by now, is another example that illustrates a more common process involving global information (Seeley, 1992b). Local cues, like wait times or encounter rates, can be used to accurately estimate global patterns, and then this information can be broadcast to the whole nest. Alarm pheromone is another example of global information sharing. An alerted guard releases a highly volatile alarm pheromone that alerts bees over a large range. Some of these bees also release alarm pheromone in a positive feedback cycle that quickly alerts the whole colony. Recent work on brood pheromone suggests that it is volatile enough to send a global signal of brood needs (Ma et al., 2018).

The way that the SO literature handles these cases is misleading. It is pointed out that the bee receiving the signal in such cases is responding to information in its local environment. This is akin to saying humans are responding to local information when they get a long-distance phone call because they are hearing sound coming from the phone in their hand, never mind that they are talking to someone halfway around the world. To return to the bees, they can and do estimate global states in many ways, often based on rules of thumb, and they also have the ability to broadcast this information to the whole nest. This organizational pattern, in general, is not solely based on local information and simple rules. Wilson and Hölldobler (1988) proposed a variant of the superorganism concept, called dense heterarchies, to account for this reliance on local and global information processing and signaling within a group of individuals in an intense communication network.

In summary, the need for SO is not due to small brains nor limited information processing ability. Humans also use SO in many cases for challenging problems, like optimal flow through telecommunication networks. In these cases, perfect solutions are too time consuming, so rules of thumb work better. The complexity of our brain has little to do with it. Workers are faced with many similar problems such that a rule-of-thumb approach involving SO works well, and any utility from a more complex neuronal mechanism would likely not be adaptive. But this may not be true in every case. It is known that foragers process an enormous amount of information about time of day, food quality, and location as they do their jobs. Hence, colony information processing is a mixture of sophisticated information processing at the individual level and self-organizing algorithms at the colony level. The two are not mutually exclusive.

Collective Behaviors

Finding a New Home

A great deal of work over the last 20 years or so has gone into understanding how a honey bee colony chooses a new home. We start with a discussion of what honey bees are looking for in a new nest and then cover how they decide which potential new nest is the best.

NEST PREFERENCES

Honey bee scouts rate the quality of the potential new nests they find. There are two aspects to this problem. The first relates to the preferences, about which much is known. The second relates to the neuroethology involved, about which little is known. Table 13.3 shows the known preferences. These were worked out in experiments with bees offered a choice between two nest boxes (Seeley and Morse, 1978). The nest boxes varied in size, shape, entrance properties, height above the ground, and so forth. All of the preferences make adaptive sense. A new nest should be far enough away to avoid competition with the old nest, but not so far away that it is challenging to get there, for example (Seeley and Morse, 1977). A nest higher up is harder to find, and one with a narrow entrance is easier to defend. Comb is costly to build, so having it already there is favored.

Turning to the neuroethology, although this has received little attention, the natural history suggests that it may be quite involved, as the scout spends about 40 minutes inspecting a potential nest (Seeley, 1977). During this time, she engages in a stereotyped pattern of movement, starting at the entrance and then working her way inside. Given that it is now known that insects can calculate distance based on number of footsteps (Collett et al., 2006), the long walk may allow for the calculation of the cavity's volume, but undoubtedly other information is processed as well.

NEST SITE SELECTION

Lindauer (1955) noticed that bees use the waggle dance on swarm surfaces and correctly surmised that they were advertising nest site locations rather than food sources. He then conducted studies that followed the decision-making process of the swarm. He found that only a few hundred scouts participate in the decision-making process, and they slowly reach a consensus

Table 13.3. Nest preferences of honey bees

Property	Preference	Function
Size of entrance	12.5–75 cm^2	Defense
Direction of entrance	South > north facing	Thermoregulation
Entrance height	5 > 1 m	Defense
Entrance position	Bottom > top	Thermoregulation
Shape of entrance	Circle = vertical	None
Cavity volume	10 < 40 > 1000 L	Storage space / thermoregulation
Comb present	Present	Energy saving
Shape of cavity	Cubical = tall	None
Dryness	Wet = dry	Bees can waterproof
Draftiness	Drafty = tight	Bees can seal holes

Source: Seeley, 2010.

after initially advertising many sites. Seeley and Visscher, several decades later, picked up where Lindauer left off and in a series of experiments greatly increased our understanding of this process.

Seeley and Buhrman (2001) began by showing that colonies are good collective decision makers. When confronted with boxes varying in quality (based on the previous studies of nest preferences), the bees almost invariably chose the better site. Attention was then turned to the mechanism by which this is accomplished. Scouts initially go out and find all the options, which they individually rate (Seeley and Buhrman, 1999, Janson et al., 2007). This rating is translated into a number of waggle dances performed on the swarm. A good site might get 50 dances from a scout upon her initial return, for example, while a less desirable site might only get 10. After advertising her site, the scout returns to the site to check on things there. As we will see later, she is likely checking up on the success of recruitment to her site. She then returns to the swarm and advertises again, but this time with less enthusiasm (Seeley, 2003). If she did 50 waggle runs the first time, she might only do 35 the second. This process repeats until the scout stops advertising her site and drops out of the decision-making process (figure 13.3). In the meantime, recruits are visiting each advertised site and are making their own assessments, which leads to more advertising. The popularity of each site is reflected in the number of scout bees performing dances for it on the surface of the swarm. Over time, the bees almost always reach a consensus (the mechanism of which is dis-

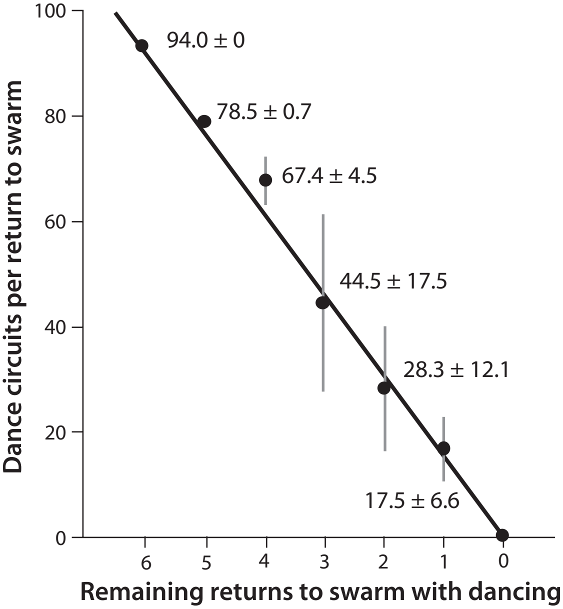


FIGURE 13.3. Decrease in the rate of advertising, per visit, via the waggle dance of a new site by a scout during the house hunting process (*from Seeley et al., 2006*).

cussed below), and the swarm takes off to the new nest. There are rare occasions when a consensus is not reached, however, and the swarm takes off and attempts to fly in two separate directions. Seeley and Visscher triggered such indecision by giving bees the option of two identical choices of potential new nests. Swarms, in this case, split in two, with each subswarm attempting to fly in its own direction before the process was aborted and the swarm reassembled around their queen (described in Seeley, 2010, pp. 168–169).

Several studies elucidated key aspects of this process. First, how do the bees know when a consensus has been reached? They could do this by paying attention to consensus building on the swarm surface, or it could be based on the success of recruitment to the site. It turns out to be the latter. A quorum (a threshold number of bees) at the new nest is the indicator that a decision has been reached (Seeley and Visscher, 2003, 2004). Once the quorum is reached, the scouts stop waggle dancing and start producing piping signals (Seeley and Tautz, 2001, Seeley et al., 2003, Schlegel et al., 2012). These signals wake up the rest of the swarm, which has been resting and conserving energy. The swarm heats up and prepares for liftoff. The signal to take off is given when the swarm is warm enough and consists of a chaotic burst of buzz run signals (Rangel and Seeley, 2008). These are burrowing buzz runs through the swarm made by the scouts. The swarm then lifts off, and the scouts, who are the only bees who know where they are going, guide the swarm to the new nest (Lindauer, 1955, Seeley et al., 1979, Beekman et al., 2006, Schultz et al., 2008). They do this by flying at astonishing speed through the flying swarm in the direction of the new site. They essentially point out the direction to fly with these streaking flights.

An additional piece of the puzzle was discovered a couple of years after the series of experiments just reviewed. It was thought that only positive recruitment was involved in the decision-making processes. That is, scouts do not interfere with recruitment to other sites but merely advertise their own. This turns out not to be the case, as the stop signal, which is used to shut down waggle dancing (Nieh, 1993, 2010), is used by scouts to inhibit recruitment to other nest sites (Seeley et al., 2012). Scouts accomplish this by directing stop signals to bees dancing for sites other than their own during the decision-making process. They use the stop signal again to shut down all dancing once the quorum is reached by directing stop signals at all active waggle dancers. Theoretical modeling suggests that a balance of positive and negative feedback in the decision-making process leads to fewer deadlocked decisions for nearly equal potential nest sites than would be the case for positive signaling alone (Seeley et al., 2012).

A final aspect of the house hunting process regards the high value of good nest sites. Suitable tree cavities are rare resources in forests, and one might expect competition for them. This is particularly true since the swarming season is brief and multiple swarms may be on the hunt for a new home at the same time. The predicted intense competition has now been shown (Rangel et al., 2010a). When bees house hunting from multiple nests find the same potential nest, they tolerate one another until one side gets a numerical advantage, at which point the stronger side kills or drives off the other colony's scouts. The winners then post guards at the entrance to the cavity and advertise their ownership with a signal that resembles the buzz run used to signal takeoff during swarming.

Thermoregulation

Some of the best-understood colonial behaviors of bees involve thermoregulation (Heinrich, 1993, 2007, Heinrich and Esch, 1994, 1997). Bees can regulate the temperature of their nest over a range from −40°C to 50°C. The natural history of both heating and cooling has been long known, but new, interesting results continue to emerge. This is mainly because of increases in thermal imaging technology. We cover the basics succinctly and review some recent interesting studies.

WINTER CLUSTERING

One of the most idiosyncratic adaptations of the honey bee involves its method of overwintering. Insects with long evolutionary histories in cold climates hibernate to avoid winter. They either dig down into the soil and go into stasis, or they stay close to the ground and allow their bodies to partially freeze (Heinrich, 1995). This is what bumble bees do, for example. Honey bees, in contrast, are active year-round. This likely reflects their tropical origin and the preadaptation of clustering for warmth that probably evolved to allow rapid brood rearing in cooler temperatures.

The large body of older work on this topic is reviewed in Winston (1991). We give an overview here. When the temperature falls below about 18°C, the bees begin to cluster in the nest about the brood zone but near the honey (Heinrich, 1985). At about 14°C, the cluster reaches a structure that is typical of bee thermoregulatory clustering. This is a shell of inactive bees on the outside several bees thick, with all the bees pointed inward, and a core in the center where the bees remain active (Farrar, 1943, Simpson, 1961). The shell bees are basically insulation, and the bees in the core produce heat with their flight muscles when the temperature falls below a threshold. Individual bees rotate from the shell to the exterior (Esch, 1960). When the bees are not rearing young, the core is at about 21°C (Fahrenholz et al., 1989). The bees in the shell are at about 8°C. Once brood rearing begins, late in winter, the bees must keep the brood, at the center of the cluster, at a minimum of 33°C, which they do by generating more heat. This requires more honey, so if colonies are going to starve, it is typically at this time (Haydak, 1958).

WARMING THE BROOD ZONE

The brood zone must be kept between 33°C and 35°C for optimal brood growth and to prevent birth defects in the developing bees. Some work has been conducted on this warming process, but it is not as well studied as winter clustering. In general, it is known that this is not a caste-specific behavior. All nonforagers over the age of two days help heat the brood (Harrison, 1987, Stabentheiner et al., 2010). Heating takes the form of bees entering an empty cell near the brood and using their flight muscles to generate heat, which is transferred to the wax (Bujok et al., 2002, Kleinhenz et al., 2003, Fehler et al., 2007). It is the temperature of the comb, not the air above it, that is regulated (Stabentheiner et al., 2010).

COOLING THE NEST

The natural history of nest cooling is well known (Lindauer, 1952, Heinrich, 1993). The first response to rising temperature is fanning of the wings by random bees scattered throughout the nest, but particularly in the brood zone (Kuhnholz and Seeley, 1997, Johnson, 2002, Ostwald et al., 2016). If this does not result in sufficient cooling, the fanners begin to form long chains, head to tail, which start at the entrance and go deep into the hive. This creates nest-wide air currents, which increases cooling (Sudarsan et al., 2012). If this is insufficient, the colony will rapidly increase water collection to begin a process akin to sweating in mammals. The information used to inform the decision to collect more water is similar to that used by nectar foragers to control their rate of foraging. The few bees that specialize in collecting water, who typically have difficulty unloading, are now able to rapidly unload, informing them that water collection is a high priority (Kuhnholz and Seeley, 1997, Ostwald et al., 2016). Hence, they begin to dance to advertise their water sources, and water collection quickly ramps up (figure 13.4). In the meantime, the MABs (who, remember, do nearly all within-nest work other than nursing) begin to spread the now rapidly incoming water over the surface of the comb. The fanning, which has never stopped, evaporates the water from the comb, causing evaporative cooling. Bees also use their tongue-lashing behavior, which is normally used to evaporate water from nectar, to evaporate water. If all of this does not work, then most of the bees evacuate the nest and cluster outside, out of the way of those working (Johnson, 2002). At this point, fanning, tongue lashing, and water spreading are the main activities going on in the hive. As Lindauer (1952) showed, this is effective to cool down the hive at almost any temperature likely to be encountered.

CO_2 Regulation

Animals that live in crowded indoor conditions must evolve either a high resistance to CO_2 or some mechanism for ventilation. Early work by Lacher (1964) showed that bees have a CO_2 sensor in their antennae, and Hazelhoff

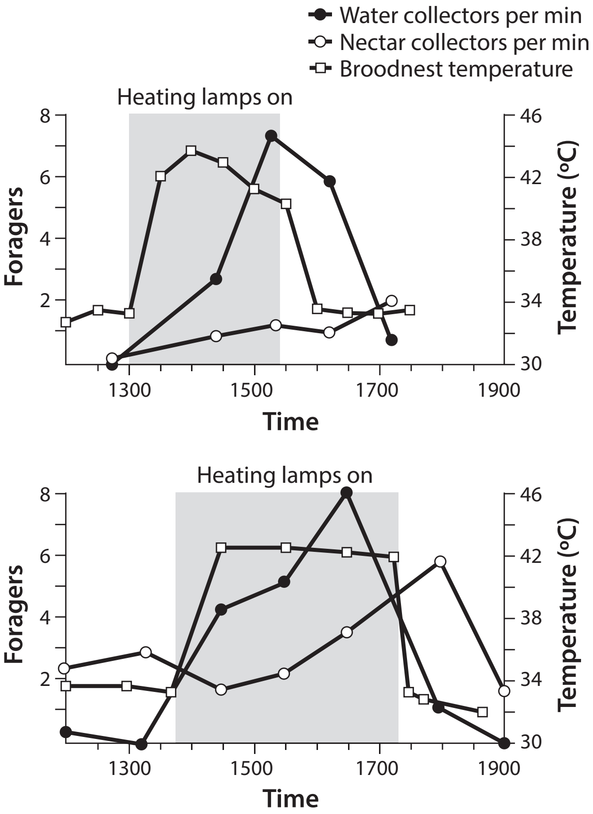


FIGURE 13.4. Basic dynamics of nest cooling illustrated by an experiment (with two replicates, top and bottom) using heat lamps to warm the brood zone. As temperature increases, the number of nectar collectors falls and the number of water collectors increases (*from Kuhnholz and Seeley, 1997*).

(1954) showed that the bees regulate CO_2 levels in the hive. A later study by Seeley (1974) slowly pumped CO_2 into a hive and observed fanning at a rate that correlated with CO_2 concentration. At the highest levels of CO_2 concentration, 40% of the population was fanning in chains to produce a stream of air leaving the hive, which quickly reduced CO_2 levels.

14

Chemical Ecology

Although the study of chemical signaling has a long history in social insects, and in honey bees in particular, it is nevertheless an understudied topic relative to its importance. Histological work on identifying glands, in particular, and chemical work on identifying the compounds eliciting behavioral responses are difficult and time consuming and, given the current notions of what constitutes “cutting-edge research,” are not rewarded as much as they should be. The result is that one of the subjects at the core of our understanding of how a colony works has many frustrating gaps. Many glands, for example, have unknown functions, and many glands with known functions have chemical bases for those functions that are unknown. Nonetheless, much progress has been made in this field since the publication of Mark Winston’s book thirty years ago.

In this chapter, we start with a review of some basic concepts in pheromonal signaling that apply across contexts. These mainly relate to how pheromones function. We then discuss the adaptive evolutionary history of pheromone signaling, exploring to what extent such signals represent honest communication between individuals in systems in which conflict has been evolutionarily resolved versus the notion that such signals represent arms races between actors with unresolved conflicts of interest. We then turn to the many known exocrine glands involved in chemical signaling and the pheromones they secrete.

Concepts in Pheromone Biology

Pheromones are chemical signals sent to members of the same species (Bradbury and Vehrencamp, 1998). Early work on pheromones sought to identify behaviors associated with chemical signaling and to determine the relevant compounds (Free, 1987). Although this is still what is basically done, the field now has a number of concepts that are useful for categorizing the different classes of pheromones (Slessor et al., 1988). Some recent work has also argued for a more aggressive reevaluation of how we think of social pheromones based on the superorganism concept (Friedman et al., 2020). We briefly cover these here, as we use this terminology in this chapter.

Primer and Releaser Pheromones

Probably the most successful concept relating to categorizing pheromones has been the notion of primers and releasers (Slessor et al., 2005). A primer pheromone is one that induces long-term changes in the recipient (Le Conte and Hefetz, 2008). This usually takes the form of changes in development, but it can also involve physiological changes relating to hormonal state or the modulation of the nervous system (Bortolotti and Costa, 2014). A releaser pheromone is one that causes a purely behavioral response in the recipient. Many pheromones are thought to have both primer and releaser functions. We cover several examples in this chapter.

Passenger and Colony Pheromones

Slessor et al. (2005) coined the expression "passenger pheromone" to account for some of the properties of the queen retinue pheromone (QRP). QRP contains the five compounds in queen mandibular gland pheromone (QMP), discussed later, plus four additional compounds found to have synergistic effects with them (Slessor et al., 1988, Keeling et al., 2003). Essentially, some components of QRP are attractive and elicit the retinue response, while other components are not attractive but are carried along. This suggests a functional reason why packaging many signals together into one secretion may be so common. Many signals can be bundled together with one "transporter" compound, which itself need not even have an effect on the receivers. Although this is a good concept that is generally important throughout the social insects, it has not caught on in the literature.

Slessor et al. (2005) also coined the term "colony pheromone" to account for the fact that some pheromones are produced by multiple bees but have the same function and the same target. Ethyl oleate, perhaps the most well understood such pheromone, is produced by larvae, the queen, and foragers to pace the rate of worker development in the temporal polyethism system (reviewed in Johnson, 2010). Essentially, information from all these sources is necessary to guide worker development, so it is natural that each group of actors would send the same signal (differing in quantity).

Pheromone Transmission

Fundamental questions in pheromone biology relate to how signals travel from sender to receiver and how they are transduced into a neurobiological or physiological response. We cover the mechanism of transmission here; the molecular aspects of detection (molecular transport, olfactory or gustatory reception) are covered in chapter 6 on neurobiology.

In general, pheromones are thought to be transmitted in three ways. First, they can be ingested, via either food exchange or some other mechanism. There was little evidence for this in the old literature (reviewed in Winston, 1991), but recent work on royal jelly showing the transfer of (E)-10-hydroxy-2-decenoic acid (10-HDA) and microRNAs breathes new life into this topic (Spannhoff et al., 2011, Wang et al., 2014, 2016). Given how poorly we understand the function of many of the compounds in brood food (worker and royal jelly), there could be several other pheromones associated with these substances. In addition, a lot of the QMP transferred from the queen to the workers in her retinue might end up in the gut (Naumann et al., 1991), meaning some of this important pheromone's action could result from ingestion. We say "might" here because Naumann and colleagues tracked QMP by following it when radioactively labeled, but it is not clear that the patterns found reflect functional QMP or degraded by-products of QMP. Brood pheromone could also be ingested, but again this is speculative. The second mechanism relates to volatile transmission. Here the pheromone travels from sender to receiver through the air and is then received via the antenna. There is, of course, much evidence for this, as many pheromones, such as Nasonov and alarm pheromone, could only function this way. Finally, the third manner relates to contact pheromones. These are oily or waxy compounds with low volatility that are thought to be transferred by direct contact, usually between the antenna or mouthparts of the receiver and the body of the sender or the nest substrate. The transmission of QRP via messenger bees seems to involve this method, as the messenger directly transfers the compound from the queen to her own body and hence throughout the nest. Early work showing the movement of radiolabeled pheromone over the body through time, along

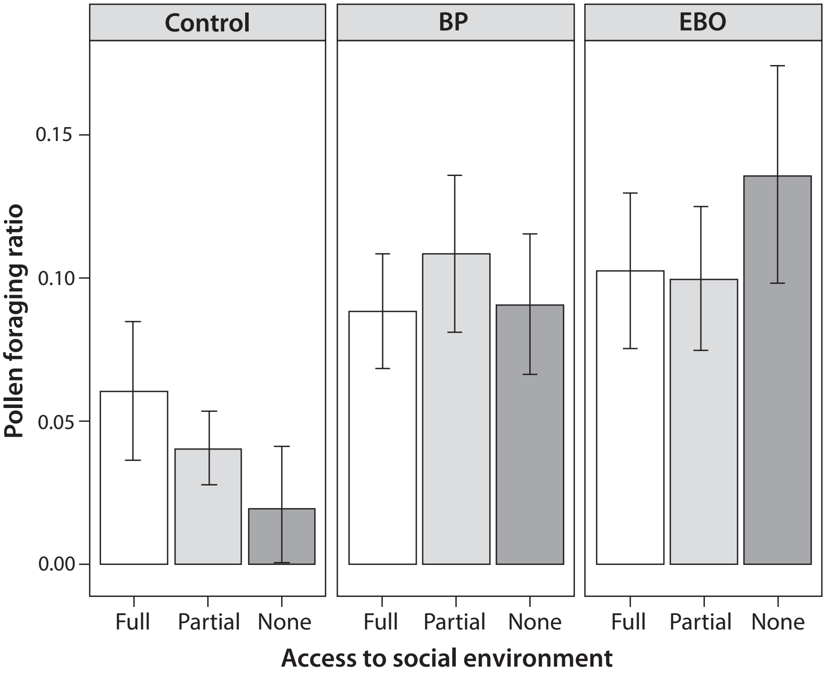


FIGURE 14.1. A double screen barring direct contact between foragers and both brood pheromones did not change the effect of either on foraging, indicating that a pheromone thought to be contact based is actually volatile (*from Ma et al., 2018*).

with work showing the attractiveness of worker body parts coming into contact with queen substance, support this view (Butler et al., 1974, Velthuis, 1972, Seeley, 1979).

A recent paper challenges some of the traditional notions of volatile versus contact pheromones (Ma et al., 2018). Brood pheromone has two main components, one thought to be a contact pheromone and the other highly volatile (Le Conte and Hefetz, 2008). An experiment designed to demonstrate this by placing both beneath the hive (not allowing direct contact) showed that both compounds have releaser effects that must be transmitted via the air (figure 14.1). Thus, there is likely no obvious degree of volatility associated with one mode of transfer or the other. Even slight volatility could be sufficient if the receiver is extremely sensitive to the compound. This might be especially true in a hot and humid nest. Thus, experiments are necessary to show that a given compound is volatile or contact based in its mode of transmission.

Social Pheromones as Social Hormones

A recent study by Friedman et al. (2020) extended the views of Wilson and Hölldobler (1988), and the general approach to pheromones based on honest signaling (Keller and Nonacs, 1993), to make some general points regarding how the study of pheromones in eusocial insects is different from that in nonsocial organisms. In general, pheromones in solitary organisms (and in simple societies) are involved in transferring information in contexts in which there is a strong conflict of interest. This limits the scope of the effects that pheromones can have. It is akin to not giving others your bank PIN number. You are willing to share some financial information with others, but there are limits that depend on how aligned your interests are with the other person. In an analogous sense, non-superorganismal insects are unlikely to allow signals from outside their body to govern their core physiological process affecting fitness (at least not without resistance).

In social insects, there is always potential conflict with nestmates, but many cases of potential conflict are resolved such that realized conflict does not occur (Keller and Nonacs, 1993). This opens the door for a massive expansion in honest signaling. Workers in the most complex colonies behave like cogs in a machine, for example. Hence, signals that would normally only be sent within a body in solitary animals are routinely sent across bodies in the social insects. Pheromonal regulators of development and physiology that are just as profound in their effects as hormones are sent from bee to bee (from queen to worker, from brood to worker, etc.). Given this fact, many, but certainly not all, social insect pheromones are analogous in function to hormones in solitary organisms and should perhaps be thought of as social hormones. This distributed physiology is quite advanced in the honey bee, in which signals from queens and larvae both regulate worker development and reproductive status (reviewed in this chapter). In a nutshell, this approach moves past the traditional argument about whether it is best to use an approach that stresses mediation of conflict (the kin selection approach), or one that stresses honest communication, to simply point out the consequences of honest communication when it provides a starting point for further evolution postresolution of the potential conflict.

Arms Races versus Honest Signaling

Many pheromones are composed of multiple compounds, some of which have unique functions while others are thought to be redundant. Two explanations for the adaptive basis of such chemical complexity have been put forward (Keller and Nonacs, 1993, Katzav-Gozansky et al., 2006, Katzav-Gozansky, 2017). The first stresses how conflict shapes behavior. Here, the reason for the redundancy is thought to be an arms race between senders and receivers. The second explanation is based on the evolution of cooperation in the context in which potential conflict has been resolved and supposes honest communication between sender and receiver. We quickly review each.

The arms race scenario posits that the queen evolves a signal that suppresses worker reproduction and the worker responds by evolving resistance to that compound (Katzav-Gozansky et al., 2004). The queen then evolves a new compound and so on. There is little explicit evidence in favor of this idea. It is mainly advanced with discussions of why it should happen in principle. Some papers claim to support it with experimental results, but these data could just as easily be explained by honest signaling (Keller and Nonacs, 1993). In general, arms races do occur in evolution, and they can lead to redundancy of responses in sender and receiver. Perhaps the arms races between males and females during mating, or those between herbivores and plants, could be used to characterize the nature of redundancy when it is due to conflict and to generate some predictions for distinguishing it from cooperation, but this has not yet been done.

Against the arms race, and in support of honest signaling, are two points. First, redundancy of signals is common in contexts in which conflict is not thought to occur. Brood pheromone is a complex signal with redundant effects between its components (Le Conte and Hefetz, 2008). It could be argued that this is because of worker-brood conflict, which could be analogous to parent-offspring conflict, but this seems a bit of a stretch. Further, alarm pheromone also has redundancy and complexity of compounds (Bortolotti and Costa, 2014). One could imagine conflicts between workers driving this, but it would be farfetched. Second, redundancy and complexity are commonplace within metazoan physiology. Endocrine signaling, neuromodulation mechanisms, and signal transduction in general are all characterized by redundancy (cross talk) and complexity of many signals with overlapping functions. Given the clonal nature of the metazoan body, this is clearly not associated with conflict of interest. Rather, redundancy and complexity are likely necessary to coordinate activities across a collective, and coordinating the workers across the superorganism poses many of the same problems. It would thus not be surprising if the same principles govern both cases of regulation of collective action.

Honey Bee Pheromones

Glands or Signals?

Most reviews of this topic take a glandular approach, stressing that gland A produces or sequesters compound B, which controls behavior C. This started with the discovery of QMP, now the most well understood honey bee pheromone (reviewed in Bortolotti and Costa, 2014). However, a discussion of honey bee pheromones that takes a glandular approach quickly becomes convoluted and difficult to follow since many responses to pheromones are caused by compounds from multiple sources. QMP itself is the prime example. A series of experiments showed that queens without mandibular glands (and queen bee extracts minus the QMP component) can still attract retinues, inhibit worker ovaries, and lead colonies for months (Velthuis and Van Es, 1964, Velthuis, 1970b, Maisonnasse et al., 2010a, Princen et al., 2019). This is true if the de-mandibulated queen is mated or not mated, or initially not mated but de-mandibulated and then artificially inseminated. These variations on the basic experiment were designed to show that it is not residual pheromones that allow the de-mandibulated queen to continue in her role. Hence, the mandibular gland, although a central source of pheromones, works in conjunction with many other pheromonal sources to control behavior, and these other sources can take the place of the mandibular gland in its absence. For this reason, it is better to organize the review by behavior and not by gland. Nevertheless, it is important to reference the glandular system, which is shown in figure 14.2.

Sexual Pheromones

Honey bee mating takes place in drone congregation areas (DCAs) high in the air and far from the nest (reviewed in Winston, 1991). How the males and females find one another has long been a question of interest. Although it is

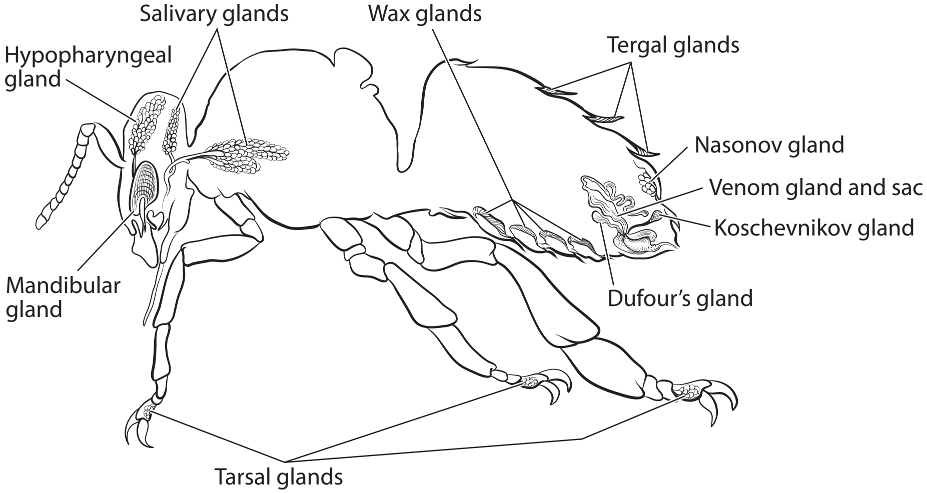


FIGURE 14.2. The exocrine gland system of the honey bee. Tergite glands are specific to the queen and the Nasonov gland is specific to the workers (*redrawn from Snodgrass, 1956*).

thought that landmark features play a prominent role, it is also known that mate finding is based in part on pheromones. Both queens and drones produce signals involved in sexual attraction, with much work having been conducted on the queen signals and little on those coming from drones.

Early studies identified QMP as important for midair attraction of drones to queens (Gary, 1962, Gary and Marston, 1971). The demonstration of this involved the use of wooden drones impregnated with queen-derived, or synthetic, compounds. Although the whole queen blend was found to be the most attractive, 9-ODA ((2E)-9-oxodecenoic acid) was identified as the major source of attraction, as a lure impregnated with 9-ODA alone was quite attractive to drones. Later work showed that the queen tergal glands also produce compounds involved in mate attraction (Renner and Vierling, 1977). Brockmann et al. (2006) dug deeper by showing that, although 9-ODA is the long-range attractant, 9-HDA ((2E)-9-hydroxydecenoic acid) and 10-HDA ((2E)-10-hydroxydecenoic acid) increase attractiveness over short distances and are probably involved in the final stages of attraction and/or mating. This was consistent with earlier work showing that 10-HDA is high in virgin queens but falls off in concentration in mated queens, suggesting it is a sexual pheromone (Plettner et al., 1997).

Regulation of Colony Reproductive State

How the queen signals her fertility and how that signal, in conjunction with other signals from the larvae, suppresses worker reproductive physiology has been a central topic of study in honey bee chemical ecology. How workers take over these roles in the absence of the queen has also received attention. We break up this work into mechanisms important in queenright versus queenless nests.

REPRODUCTIVE PHEROMONAL SIGNALING IN QUEENRIGHT NESTS—THE RETINUE RESPONSE AND DISSEMINATION OF THE QUEEN SIGNAL

A queen is typically surrounded by an oval of young workers, called her retinue (plate 14). These bees lick, feed, and groom the queen, and they spread her signal about the nest in their role as messenger bees (Seeley, 1979, Naumann et al., 1991). Bees not in the retinue are also obviously aware of the queen's presence because, as she passes by, they tend to jump out of her way. Thus, young bees are attracted to the queen, while older bees are repulsed by her. The retinue response has been the subject of more work than perhaps any other topic in honey bee pheromone biology.

Early work on the retinue response identified the queen's mandibular gland as the source of compounds involved in the response and stressed the roles played by 9-ODA and (R)- and (S)- HDA (reviewed in Winston, 1991). Conflicting results, however, plagued early research, as inconsistent retinue responses to these compounds were found across studies (reviewed by Free, 1987). This problem was finally resolved in a key study by Slessor et al. (1988). In this work, the researchers showed that the retinue response is caused by a blend of five compounds (9-ODA, HDA (both enantiomers), methyl p-hydroxybenzoate (HOB), and 4-hydroxy-3-methyoxyphenylethanol (HVA)). This conclusion was revised by Keeling et al. (2003) when they showed that four additional compounds are also involved (figure 14.3). These other compounds are not in themselves attractive, but they increase the attractiveness of the blend in QMP. Two of these other four compounds are also not produced in the head, with one thought to come from the Dufour's gland and another from somewhere in the abdomen. All nine compounds were thus called the queen retinue response pheromone (QRP), although this name did not catch on in the literature. It has also been shown that compounds from the tergal and Dufour's glands are involved in the retinue response (Wossler and Crewe, 1999b, Katzav-Gozansky

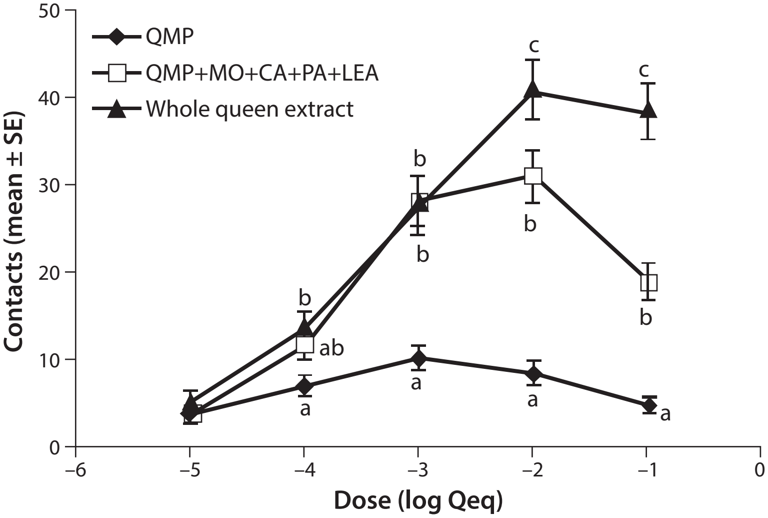


FIGURE 14.3. QRP is more effective at triggering the retinue response than QMP alone, but not as attractive as whole queen extraction (*from Keeling et al., 2003*).

et al., 2001). This work is preliminary, however, and how compounds from these sources interact with QRP is not understood.

It would not be surprising to find that yet more compounds are involved in the retinue response (Dehazan et al., 1989, Keeling et al., 2003). Further, for unclear reasons, this body of work has not built on the work using demandibulated queens. It would be useful to extract compounds from these queens to determine how they attract a retinue. Further, using live queens as controls instead of whole queen extracts in lab bioassays may be helpful, as extracts may disrupt the natural balance between compounds found in a living queen.

WORKER OVARY SUPPRESSION

Suppression of the workers' ovaries in a queenright colony is perhaps the best-understood primer effect of social pheromones. It is known that at least three glands in two different life history stages of bees produce compounds having this effect, with several having known chemical structures. That the pheromones should come from two bee sources is quite predictable, given the functions they serve in reproduction. As we reviewed in chapter 8, workers only activate their ovaries when two things are true: the queen is gone and there are no young eggs from which to rear a new queen. Thus, we should expect signals from both the queen and the young brood, and this is what has been found.

Early work on queen signals showed that components of QMP inhibit ovary development but that they are not as effective as extracts from the whole queen (reviewed in Free, 1987). Later work, just as for the retinue response, showed that the blend of compounds found by Slessor et al. (1988) strongly inhibits worker ovaries. The additional four compounds found to be involved in the retinue response (Keeling et al., 2003, Hoover et al., 2003) do not appear to be involved in ovary suppression. With respect to brood pheromone (BP), early work showed that workers are more likely to activate their ovaries in the absence of open brood, suggesting a signal emanating from this source (Kropacova and Haslbachova, 1970). Later work showed that extracts from brood inhibit worker ovaries (Le Conte et al., 1990, Arnold et al., 1994, Mohammedi et al., 1996, 1998). Ethyl palmitate and methyl linoleate, in particular, were shown to have this effect, but the whole blend is probably more important (Mohammedi et al., 1998). Recent work has shown that a highly volatile component, E-beta-ocimene, produced at high levels by early larval instars, inhibits worker ovaries, probably as a distinct component relative to the other elements in BP (Maisonnasse et al., 2009, 2010b). Finally, extracts from the queen's tergal glands also have primer effects, inhibiting worker ovary development (Wossler and Crewe, 1999a). As for the many other behaviors associated with tergal gland pheromone, the compounds have not been described, and little work has followed up on the preliminary studies.

REGULATION OF REPRODUCTIVE PHYSIOLOGY IN QUEENLESS COLONIES

As we reviewed in chapter 9, a colony does not break down into anarchy when the queen dies and a new one cannot be reared. Rather, some bees activate their ovaries and lay male eggs, while most of the bees continue as workers, collecting and processing food and rearing the young. Activities such as building new comb, or other tasks that would be futile in a dead-end colony, are not continued. Thus, there must be signaling to regulate the activities of the permanently queenless hive in a manner that will optimize its efforts for maximizing drone production.

The pheromones that regulate behavior and development in queenless colonies have received considerable attention, but due to the complexity of the issues involved, this phenomenon is poorly understood. The main problem is that there are at least three distinct contexts in which colonies are queenless. These are normal wild-type colonies that have lost their queen and have no young brood, Cape honey bee queenless colonies, and anarchistic colonies. One cannot assume that the mechanisms that govern queenlessness (or worker egg laying) in any of these contexts are relevant to any of the others, as they represent distinct biological phenomena. Capensis workers vary because of local adaptation (a unique evolutionary history), while anarchistic bees can be thought of as having a rare and destructive (to colony fitness) genetic disease. We discussed the reproductive biology of the Cape honey bee and anarchistic bees in chapter 8; here we focus on typical permanently queenless colonies.

Early work showed that queenless colonies have a variable number of egg-laying pseudo queens. These are defined as workers who attract a retinue (Sakagami, 1958). Later work showed that the mandibular glands of pseudo queens produce a queenlike blend of pheromones (Plettner et al., 1993, Tan et al., 2012). Later work also showed, however, that not all laying workers are pseudo queens. In fact, the best indicator of fertility in the workers is the activity of the Dufour's gland, which begins to produce normally queen-specific esters when a worker activates her ovaries (Katzav-Gozansky et al., 1997, 2004, Sole et al., 2002, Dor et al., 2005). There is no correlation between queen-like pheromone production in the mandibular and Dufour's glands. Hence, a pseudo queen only has some chemical characteristics of a queen. Finally, drone larvae produce a pheromone that also suppresses activation of worker ovaries and leads to cessation of egg laying, although this has not received much attention from a chemical ecology perspective (Sakagami, 1958, Velthuis et al., 1965, Velthuis, 1970a, Jay and Nelson, 1973).

FERTILITY SIGNALING

A series of studies have shown that the Dufour's gland produces a queen-specific blend of esters (Katzav-Gozansky et al., 1997, 2004). In queenless nests, the same compounds seem to be a fertility signal of laying workers, so it stands to reason that they play the same basic role in queens, albeit modified to fit a queenright context. As we saw in chapter 8, honey bees have a number of distinct colony-level reproductive processes. Swarming has been explained to some degree, but what is missing is an explanation for supersedure. This is when an older, less fertile queen is replaced. This is distinct from swarming in many ways, including the number of new queens reared and a lack of preparation for moving. A fertility signal is precisely the sort of information the bees would need to make the decision to replace their queen. Unfortunately, whether lack of a fertility signal from the Dufour's gland leads to supersedure has not been explored.

Brood Capping

In addition to its role as a primer pheromone, BP also has several releaser functions. One is to trigger capping of brood cells. Several components of BP—methyl palmitate, methyl oleate, and methyl linolenate—have been shown to peak in production when larvae are ready to be capped (Le Conte et al., 1990, Trouiller et al., 1991, Qin et al., 2019).

Swarming Pheromones

Chemical signaling underlies the decision to swarm, the production of new queens, and the behavior of the issued swarm. This work primarily stems from the older literature, as this important topic has not received much attention in recent years.

Swarming Initiation

How a colony determines when to swarm is a major question for honey bee biology. Surprisingly, although there has been endless speculation about this process, it is not well explored experimentally. This may be a good place to explain such an apparent contradiction because it is a good example of a broader phenomenon that affects the study of many important honey bee traits. The cause of this imbalance between the importance of the topic and the paucity of work on it relates to swarming being a honey bee–specific trait (really, restricted to a small number of social insects). Thus, one cannot claim to be studying something foundational to biology by studying this trait. In general, it is difficult to get serious funding to study bee-specific traits because the funding agencies want research that is broad and applicable across the tree of life. Given this, it would be beneficial for bee-specific funding sources to prioritize the study of bee-specific traits like swarming.

We reviewed the basic biology of swarming in chapter 8. The main issues, remember, are crowding in the nest (or population size) and time of year.

These are clear, given that bees will swarm almost any time of the year if they outgrow their nest, but in general swarming tends to be a seasonal process. Hence, there is likely some general mechanism in place that triggers swarming, which is modulated by time of year. The simplest manner in which this could occur is by changing the workers' response thresholds to the relevant stimuli. Although this is an obvious hypothesis, it has not been tested. With respect to the roles of pheromones in this process, there are a few key studies. Naumann et al. (1991) showed that QMP travels effectively throughout the nest (measured with radiolabeled QMP) in both crowded and uncrowded conditions. The amount of signal to reach workers was found to mainly be a function of population size. This argues against a simple model assuming that crowding diminishes the spread of QMP in the nest. The best study to date was conducted by Lensky and Slabezki (1981). They showed that both QMP and queen tarsal gland pheromone together inhibit the production of new queens. Either QMP or tarsal gland pheromone alone was insufficient. A simple explanation is that, in a crowded nest, the queen is able to cover less ground as she walks. When no cells are available to lay in, she may further slow down her movement rate. Thus, although QMP travels quickly (via the messenger bees), the footprints of the queen are a function of her activity, which can be predicted to change when the colony has outgrown its nest. One can then suppose that worker response thresholds to these stimuli during the appropriate season are more favorable to queen rearing than they are during periods when swarming is less favorable.

Queen Cup Regulation

A problem that arises during swarming is how many new queens to rear. There is strong variation in this trait across subspecies, with some raising only a handful of new queens and others a great many (Winston, 1991, Hepburn and Radloff, 1998). Regulation is thought to depend on queen pupae producing a chemical signal. Le Conte et al. (1994a) identified this signal, which contains methyl oleate, methyl palmitate, and ethyl oleate. As these compounds are used in other contexts, their meaning must be context specific and must depend on concentration ratios or on the presence of other compounds in the queen pupa signal. In general, that the same compounds are used repeatedly across contexts is simply an example of natural selection making use of what is present at the time selection begins to operate. A great variety of compounds already produced can be modified more simply than wholesale new compounds can be produced (particularly given that they need receptors), so it should not be surprising that social insect pheromones exhibit complexity and redundancy.

Swarm Formation and Cohesion

Pheromones are involved in controlling the behavior of the swarm once it leaves the hive. The clustering of the swarm on a tree branch is facilitated by the presence of the queen and is signaled by both 9-ODA and 9-HDA (Morse, 1963, Butler et al., 1964, Winston et al., 1982). These are both part of QMP and, as predicted, de-mandibulated queens cannot attract a swarm (Velthuis and Van Es, 1964). 9-ODA seems to be the long-distance attraction signal, while 9-HDA helps stabilize the cluster, perhaps by inducing workers to release Nasonov gland pheromone (Bortolotti and Costa, 2014).

Nasonov pheromone is produced by the Nasonov gland in an unmistakable behavior in which the gland is everted on a raised abdomen accompanied by fanning of the wings to disperse the highly volatile scent. It is a homing beacon used in many contexts. It has a lemongrass smell and is a complex blend of compounds. However, because the compounds in the Nasonov pheromone are biochemically related, the signal may represent a single message with the diversity of compounds produced due to chemical constraints (reviewed in Winston, 1991).

Pheromones Regulating Temporal Polyethism

Regulating division of labor is a central function of pheromones. With respect to nursing behavior, BP has been shown to activate (prime) the hypopharyngeal glands for the production of brood food (Mohammedi et al., 1996, Pankiw et al., 2008, Sagili and Pankiw, 2009). QMP has been shown to modulate dopamine levels in the brain (Beggs et al., 2007, Vergoz et al., 2007b, Beggs and Mercer, 2009). This may involve decreasing movement rates, or perhaps fine-tuning the cognitive functions of nurses in a manner that specializes them for their limited task repertoire (Johnson, 2010a). QMP and BP also both suppress JH titer (a low JH titer is characteristic of nursing), which may be associated with turning off all the many pathways associated with nonnursing tasks (Kaatz et al., 1992, Le Conte et al., 2001, Grozinger et al., 2007).

With respect to the later DOL transitions, ethyl oleate has been shown to regulate the transition to foraging (Huang and Robinson, 1996, Leoncini et al., 2004). This pheromone is produced in the forager's crop and is present on the cuticle of the bee's body (Castillo et al., 2012). Levels of this pheromone correlate with forager population levels (and foraging activity) and are thus a good information source for within-nest bees. Pankiw et al. (2004b) provided evidence that a pheromone with a similar function (regulating the rate of development related to DOL) may be produced by nurses, as whole-body extracts of nurses decrease the age at first foraging. The same study showed that forager extracted compounds increase the age at first foraging. Thus, high levels of a forager pheromonal "pacer signal" may mean that sufficient foragers are present, thus lowering the transition into this case, while high levels of nurse pheromonal "pacer" may signal a high demand for pollen foraging and hence an earlier than normal transition to foraging. This is speculative, but follow-up work on Pankiw's study of nurse-produced social pheromones is long overdue.

Foraging-Associated Pheromones

There are a number of pheromones with releaser effects on various aspects of foraging. BP, both E-beta-ocimene and the 10-component blend with less volatility, stimulate pollen foraging (Pankiw et al., 2008, Ma et al., 2018). The adaptive function is rather obvious, as a signal from the brood that quantifies their protein need is clearly useful for modulating foraging effort. Older larvae, which are orders of magnitude larger than young larvae, secrete more BP (Le Conte et al., 1994b, 1995). Recent work has shown that the waggle dance has a pheromonal component (Thom et al., 2007). Dancers release a volatile pheromone containing at least three cuticular hydrocarbons (CHCs)—(Z)-9-tricosene, ticosane, and pentacosane—that together activate experienced foragers and increase the level of dancing in the colony (Gilley et al., 2012, Gilley, 2014). With respect to marking of high-quality forage sites, the Nasonov gland plays a poorly understood role (reviewed in Winston, 1991). Foragers release Nasonov gland pheromone at particularly high value forage sites, such as feeders. It is not clear how often they do this at natural nectar sources, however. They also release Nasonov pheromone at water sources, a more obvious function, since water—unlike flowers—does not have an odor that would help in locating it. Finally, the function of 2-heptanone, which is produced by the worker mandibular glands, has long puzzled scientists (Shearer and Boch, 1965, Balderrama et al., 2002). It was initially thought to be an alarm pheromone, but there are no clear data to support this. One possible role may be as a flower avoidance signal (Simpson, 1966). This compound is on the mandibles, which come into contact with flowers during foraging, and studies have shown that adding 2-heptanone to flowers makes them less attractive to bees (Vallet et al., 1991). This pheromone may thus be how honey bees mark depleted flowers, an ability of most bee species (Giurfa and Nunez, 1992, Giurfa, 1993).

Alarm Pheromones

Alarm pheromones were some of the earliest pheromones discovered. Alarm pheromone is produced by the Koschevnikov gland along with glandular tissue near the sting sheath (Cassier et al., 1994). The resulting secretions exit at the base of the sting onto a large number of setae, which create a large surface area for volatilization. Isopentyl acetate (IPA) was identified early as the major constituent of the pheromone (Boch et al., 1962, Boch and Shearer, 1966, Blum et al., 1978). Considerable work has taken place since these early studies (Bortolotti and Costa, 2014). Much of it explores the neurobiology of aggression. Here we limit ourselves to more traditional questions in chemical ecology.

The manner in which work on alarm pheromone is typically reviewed is curious in that a deference to past results seems to characterize discussion to a degree not seen in similar contexts. Early work on queen signal, for example, exaggerated the role of 9-ODA, while later work showed that, although this compound is important, it is only part of a blend of important compounds. The story of alarm pheromone is similar. Although IPA is typically discussed as though it is the alarm pheromone, in reality IPA by itself is not nearly as effective at eliciting a response as the whole sting gland extract (Boch et al., 1962). Pickett et al. (1982) resolved some of the issue by showing that (Z)-11-eicosen-1-ol is also present at high levels in the alarm pheromone and has a strong effect on stinging in *A. mellifera*, although not in other *Apis* species (Martin et al., 2007). IPA plus (Z)-11-eicosen-1-ol is as effective as whole sting gland extract in some bioassays (figure 14.4). Thus, sting pheromone is a blend of at least two compounds in *A. Mellifera*, with IPA playing a long-distance attractant role and (Z)-11-eicosen-1-ol a role closer to the skin. This pattern of volatile pheromones having distinct components playing long- and short-range roles has recurred throughout our discussion of pheromones and is probably a general principle. Many other compounds may play a role in alarm pheromone since a great many compounds are present, and

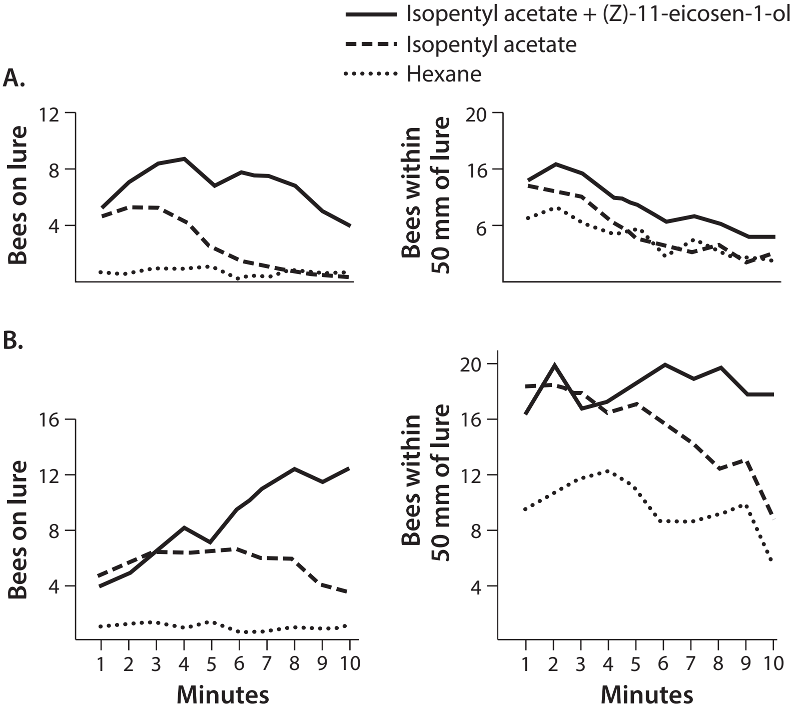


FIGURE 14.4. (Z)-11-eicosen-1-ol does not affect long-distance attraction, but in combination with isopentyl acetate increases contact with the lure, suggesting that it is a close-distance attractant associated with stinging behavior. (A) 500 micrograms of test chemical. (B) 5000 micrograms of test chemical (*from Pickett et al., 1982*).

many of these have been shown to have physiological effects on bees (reviewed in Free, 1987).

Finally, it is not the case that alarm pheromones alone cause stinging. Visual cues are probably just as important as chemical ones for eliciting stinging. The pheromones may rather act like adrenaline to cause a fight-or-flight response at the physiological level (Nunez et al., 1997, Alaux and Robinson, 2007), in addition to aiding in finding the target. This may involve activation of metabolic and other pathways involved with excitation in older bees and the opposite in younger bees (Collins, 1980, Collins et al., 1980). This context-dependent nature of the signal relative to its receiver (the need to send more than one message) may be associated with the complexity in the signal.

Nestmate Recognition

Basic Concepts

The ability to distinguish self from non-self is one of the many common needs of cells in an organism and individuals in a superorganism. This ability is thought to be based on a process that is simple in principle but complex in application (Crozier and Dix, 1979, Lacy and Sherman, 1983, Breed, 1983, Grosberg, 1988, Reeve, 1989). In short, the actor making the decision is thought to compare the recognition labels on an individual they encounter with a set of labels they contain. If there is a match, then the individual is accepted, while a mismatch triggers rejection. The labels contained by the actor are often called the template. The process is sometimes broken up into three phases: the expression phase, the perception phase, and the action phase (Tsutsui, 2004). The expression phase deals with the labels themselves (their form and ontogeny), the perception phase deals with the neurobiology relating to the comparison of the labels to the template, and the action phase involves the response. We focus on phase one.

What Labels Do Bees Use in Nestmate Recognition?

The study of nestmate recognition is a curious one in that most of the critical early work was on wasps and bees, but later work on ants came to be central to our current understanding (van Zweden and d'Ettorre, 2010, van Wilgenburg et al., 2011). Essentially, the model found to work for ants, which was initially inconsistent with earlier work on bees, has been strongly supported by recent studies.

CRITICAL EARLY STUDIES

Early work on honey bee nestmate recognition ruled out some hypotheses, found strong support for other hypotheses that remain uncontested, and found support for some hypotheses that later work has not supported. The earliest work showed that external sources of odors, such as floral odors, do not play a role in nestmate recognition (reviewed in Breed, 1998). Work also suggested that recognition labels may not be present on newly emerged bees but that they are acquired after a few days in the nest (Breed et al., 1988a, 2004b). A large body of work also showed that the comb mediates nestmate recognition by acting as a sink for the necessary labels. This was shown in studies demonstrating that adding comb from a foreign nest to a colony makes bees from that foreign colony more likely to be accepted (Breed et al., 1988b, 1995, D'Ettorre et al., 2006, Couvillon et al., 2007).

With respect to the chemicals used as labels, it was shown that the cuticle contains many classes of compounds, including esters, alcohols, free fatty acids, and hydrocarbons (Breed et al., 1988b, 2015, Breed and Stiller, 1992, Breed, 1998, Kather et al., 2011). The hydrocarbons were either alkanes or alkenes with methyl groups at variable positions. The question was, Which of these compounds are the labels? Breed, in a series of studies using bioassays in which bees in the lab were treated with compounds and then allowed to interact with other bees, showed that the addition of esters, fatty acids, and alkenes can trigger aggression (Breed and Stiller, 1992, Breed et al., 1992b, Breed, 1998). Breed concluded that fatty acids, in particular, play a prominent role. Later work, particularly in ants, has stressed the role played by alkenes and argued against a role for fatty acids and esters, so it will be worthwhile to spend some time considering this issue.

Although a definitive answer cannot be given as to whether fatty acids play a role in nestmate recognition, there is a simple alternative explanation for the disagreement between those advocating for fatty acids or alkenes. The cuticle contains many compounds, some of which are used for nestmate recognition but many of which have other functions. A whole-body extraction is going to capture all of these signals. A key thing to consider is thus whether the bioassay used in a nestmate recognition study can be confounded by signals with other purposes. In the case of esters and fatty acids, these are known to be involved in reproductive signaling by the queen and by pseudo queens (reviewed in Le Conte and Hefetz, 2008). Adding fertility signals to a worker's cuticle and then exposing the worker to nestmates may thus trigger aggression for reasons wholly unrelated to nestmate recognition. In this light, the work of Breed and coauthors (2015) showing that the addition of various esters and fatty acids triggers aggression cannot unequivocally be interpreted as supporting a role for these compounds in nestmate recognition, as those bees could have been attacked for false fertility signaling (Katzav-Gozansky et al., 1997, 2002). Given that later work does not support the notion that there is sufficient variability between colonies in these compounds (Kather et al., 2011), and the alternative explanation for why they might trigger aggression in nestmate recognition bioassays, it will be necessary to conclude here that esters and fatty acids are unlikely to be involved in nestmate recognition in honey bees. This could be wrong, of course, but perhaps stating this so forcefully here will cause

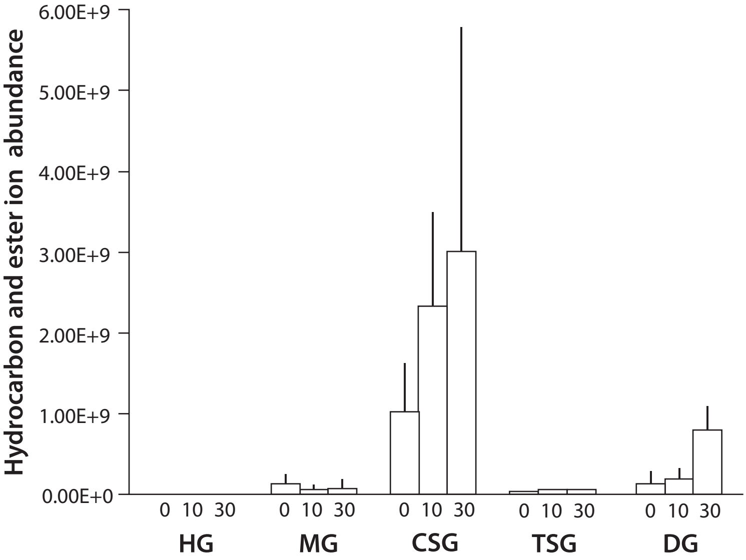


FIGURE 14.5. Amount of oleic acid wax esters and CHCs in the major honey bee glands at three ages (0, 10, 30 days). Abbreviations: Hypopharyngeal gland (HG), mandibular gland (MG), cephalic salivary gland (CSG), thoracic salivary gland (TSG), Dufour's gland (DG) (*from Martin et al., 2018*).

those who advocate for the inclusion of these compounds as labels to conduct more complete experimental studies demonstrating their role.

ALKENES AS HONEY BEE LABELS

Breed showed that alkenes were potential nestmate recognition labels (Breed and Julian, 1992, Breed, 1998), and later work has strongly supported this and expanded our understanding of the relevant biology. First, it was shown in a more natural context than in past experiments that the addition of alkenes, but not alkanes, to the cuticle triggers rejection (Dani et al., 2005, Martin et al., 2008). The same year, it was shown using the PER bioassay that bees are better able to learn alkene odors relative to alkane odors (Chaline et al., 2005). Two recent studies then really clarified the question. First, Pradella et al. (2015) used recognition errors (incorrect acceptance or rejection decisions) to determine what compounds are associated with these mistakes. They showed that four alkenes, eicosane, (Z)-10-hentriacontene, (Z)-8-hentriacontene, and x,y-tritriacontadiene, explain much of the variation in rejection and acceptance errors. These compounds have also been shown to be mimicked by bee parasites (in bumble bees, who also seem to use these compounds for nestmate recognition) and by *Varroa* (Martin et al., 2010, Kather et al., 2015). Second, Martin et al. (2018) showed that the cephalic salivary gland produces CHCs of many sorts, including the key alkenes in great abundance (figure 14.5). These compounds are worked into the comb during comb production (and probably over time) and are also present on the bee's cuticle. Ants, for whom alkenes were shown to be the key compounds a few years prior to this work in bees (van Zweden and d'Ettorre, 2010), have a gland, the postpharyngeal gland, that produces their labels, and the lack of such a source for bees was a critical missing link. The current model for bees is thus much like the one with the most support in ants, moving the field toward a unifying conceptual model of nestmate recognition in the social insects.

15

Foraging

Our understanding of foraging in honey bees has radically expanded over the last several decades. Large bodies of work now exist for many topics, all of which are interrelated. We break up our discussion into three sections. We start with, and primarily focus on, the impressive body of work on collective foraging. This is a colony-level exploration of food collection, both its mechanisms and adaptive bases. We then turn to studies of the individual-level biology of foragers. This includes traits underlying collective foraging and also some work on those aspects of foraging that honey bees share with all bees. We conclude with a brief discussion of perhaps the most pressing unresolved problem, which is how the collective and individual-oriented work tie together.

Collective Nectar Foraging

The waggle dance is one of the most well-known signals in the animal kingdom, and for non–honey bee biologists it is easy to assume that it underlies nectar foraging in a straightforward manner. In reality, there is a dance language of multiple signals that regulate interrelated variables associated with nectar collection and processing. It is thus useful to begin with an overview of the problem before jumping into what we know of collective foraging behavior.

Foraging is a partitioned task in honey bees (Seeley, 1986). Both the foragers and the middle-age bees (MABs) are equally involved, and foraging cannot proceed without flexible responses from both groups (Seeley, 1989a, 1989b). In particular, there are three activities that must be kept in balance. First, the foragers must find the food and optimally allocate themselves to floral patches in the field. This has received the most attention (Seeley et al., 1991). Second, nectar is transferred from foragers to receivers (members of the MAB caste) on the dance floor (Seeley, 1989b, 1992b, Seeley et al., 1996). Receivers process the nectar into honey and store it (mostly at the top of the nest). This is a time-consuming process and thousands of bees are typically involved. Third, MABs must ensure that sufficient comb space is always present. This involves wax secretion and comb building, other energetically costly and time-consuming activities (Pratt, 1998a). If any of these activities is insufficiently performed, a breakdown in the work rate of the others ensues. The causes are straightforward. The foragers cannot collect more nectar if their crops cannot be unloaded. Likewise, the nectar processors cannot unload honey if there is no room in the comb, something that can happen quickly during a nectar flow.

Allocation of Foragers to Patches

Our understanding of collective foraging in honey bees has a long history. In general, von Frisch, and the other early researchers, identified all the relevant signals and hypothesized how they might work to control foraging (von Frisch, 1967). Seeley and his coauthors, along with other contemporary researchers, experimentally tested and refined these hypotheses (Farina and Nunez, 1991, Waddington et al., 1994, Seeley, 1995, Beekman and Ratnieks, 2000, Dornhaus and Chittka, 2004). They also created new hypotheses for aspects of the problem unknown to earlier authors, or for topics on which von Frisch was incorrect. For Seeley, this involved a long series of studies lasting about two decades, culminating in one of our best-understood case histories for collective behavior in insects (reviewed in Seeley, 1995).

We provide a summary of the process by which colonies allocate foragers to flower patches, but the interested reader is encouraged to read *The Wisdom of the Hive* (Seeley, 1995) for a full treatment. Scouts explore the area around the colony up to a radius of several kilometers, although this varies strongly with environmental conditions (Seeley, 1986, 1987, Waddington et al., 1994, Beekman and Ratnieks, 2000). Scouts evaluate the nectar they find as high or low value and perform waggle dances on the dance floor that reflect this decision (Seeley, 1985b, 1986, 1987, 1989b, Seeley et al., 1991). This means more waggle runs for better sites. The result is that the sites worthy of being reported are advertised on the dance floor according to their value. This process thus translates all the relevant environmental information (distribution of floral patches with their respective values) into an information well (or billboard) from which foraging recruits can sample. If there are three patches available, for example, and they rank in value from one to three, then this is reflected by the number of dances being performed for each site on the dance floor. As long as recruits sample from this information well at random (choose dancers to follow at random), which they do, then the allocation of recruits to patches will match the distribution of values assigned to those sites by the bees dancing for them (Seeley and Towne, 1992, Bartholdi et al., 1993). Hence, although each forager only evaluates one patch, and knows nothing of the global distribution of nectar, the colony evaluates all flower patches within flight range and collates the resulting information into a usable form for recruits. This can lead to an optimal allocation of foragers to sites (Seeley et al., 1991, Seeley, 1995).

The allocation of recruits to patches by this system is a dynamic equilibrium, meaning that it can track many kinds of change without requiring new behavior from the bees. Each recruit, for example, assesses patch quality and either dances, forages without dancing, or quits, depending on this assessment. Hence, allocation to a patch reflects its current quality, and the colony state. The system therefore leads to either recruitment or abandonment of sites. Thus, when conditions change, the allocation of bees to patches changes with it. Several modeling papers, based on experimental work, have explored this basic algorithm (Seeley et al., 1991, Camazine and Sneyd, 1991, de Vries and Biesmeijer, 1998).

It is important to stress that this system of allocation does not reflect all the foraging going on in a colony for nectar. Scouts work patches not profitable enough to trigger waggle dancing but good enough for a single bee to continue visiting (Seeley, 1983, Biesmeijer and Seeley, 2005, Beekman et al., 2007). Bees also have amazing memories for past patches, now run dry, and they make use of these memories when deciding whether to look for new sites or return to old ones (von Frisch, 1967, Farina et al., 2005, Biesmeijer and Seeley, 2005). A bee that smells the odor of a recently visited flower patch on the dance floor will return to that site with or without following dances for it. Honey bee foraging is thus a complex mixture of sophisticated individual-level decision making and higher-level collective behavior.

Collective Decision Making under Natural Conditions

An important caveat to the previous section is that our discussion was of a special case; in other words, the optimal allocation of bees to patches is one particular outcome of honey bee collective foraging. The algorithm (scouts find all the sites and the colony allocates to each in accordance with quality) is likely dependent on the distribution of patches outside the nest and how that relates to the size of the colony's workforce. When patches are few and foragers are many, colonies can easily find all the patches and allocate optimally. As Waddington et al. (1994) found, however, in a different setting (suburban environments where there are many small but valuable patches), colonies do not always find all patches (or even come close to it), and they may or may not allocate in accordance with optimal efficiency. In this context, the environmental information is too cumbersome for the colony to conduct optimal decision making, although they still make adaptive decisions that favor better over worse sites.

To build on the now classic work reviewed in *The Wisdom of the Hive,* future work will have to determine the nature of natural floral distributions in a variety of habitats (both in the native range and in novel environments), the likelihood that they are found by a colony's scouts (controlling for colony size), and the colony density in the relevant habitat. This will involve transitioning from a primarily behavioral ecology approach to one that places equal emphasis on ecology and evolution.

Coordinating Activity within the Colony

As we reviewed in chapter 13, there are several signals known, or thought, to be involved in regulating nectar foraging, receiving, and comb building. The tremble dance, produced by foragers in two contexts, is associated with boosting, or maintaining, levels of nectar receiving, although it may have other functions as well (Seeley, 1992b, Kirchner, 1993b, Seeley et al., 1996, Anderson and Ratnieks, 1999). Likewise, the shaking signal modulates general within-nest activity, something central to foraging. After a dearth, for example, Seeley et al. (1998) showed that the first dances produced by foragers after finding food are shaking signals, presumably to reactivate nectar receiving and processing. The worker piping signal is also produced within the nest, and this may also be due to changes in foraging conditions (Pratt et al., 1996, Thom et al., 2003). What this signal does in this context, however, is not understood. In general, coordination of foraging with within-nest activities is incompletely understood for reasons discussed in the section on modulatory signaling in chapter 13.

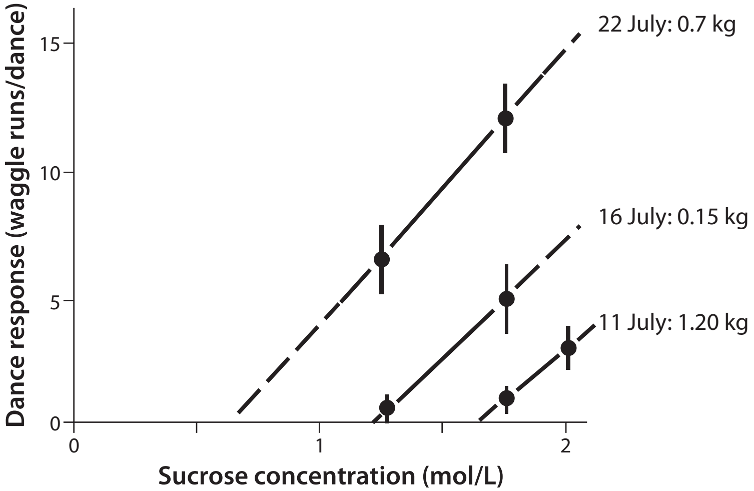


FIGURE 15.1. Probability of waggle dancing for the same-quality sugar water offered at a feeder on three dates. Natural food sources were plentiful on July 11 but scarce on July 22 (*from Seeley, 1994*).

Forager Assessment of Patch Quality

A core component of the mechanism allocating foragers to patches is the ability of bees to rate the quality of a site. This is thought to involve two separate abilities, which are integrated into one decision. First, bees are assumed to have an innate ability to rank floral quality. This is thought to mirror their ability to instinctively rank potential nest sites (Seeley, 1985b, 1986, 1987, Seeley et al., 2000, Seeley and Buhrman, 2001). The likelihood of dancing cannot, however, be based solely on the evaluation of nectar quality made in the field, because the decision varies with conditions. Figure 15.1 shows that a food dish presented to bees with 1.75 M sugar water elicited weak dances on July 11 (when food was plentiful) but strong dancing 11 days later when food was scarce (Seeley, 1994). Bees are thus able to modulate their innate ranking in accordance with colony needs.

The mechanism by which foragers integrate the colony's needs with their assessment of patch quality is based on cues the foragers experience as they try to unload their nectar. As we reviewed previously in chapter 13, a forager uses the wait time to find a receiver bee to inform her of the current balance between nectar influx and receiving capacity (Seeley, 1989b, Seeley and Tovey, 1994). A long wait time means there are too few receivers, while a short wait time means there is superfluous labor among the receivers and more capacity for nectar processing. This information is used to weight the probability and duration of dancing, as it is maladaptive to increase foraging when receiving levels are insufficient, but dancing should be vigorous if the colony can process more nectar. Thus, a bee returning from the field and experiencing a short wait time is more likely to waggle dance for the same site than if she had experienced a long wait time.

Scouting

Scouting, and the individual-level foraging that it often leads to, is much like the foraging of solitary bees. This is interesting because it suggests that honey bees likely retain many of those foraging abilities present in solitary bees, in addition to those associated with collective decision making. This is a clear example of the expansion of behavior associated with the evolution of eusociality, which may be associated with expansive changes at the genomic level (Oster and Wilson, 1978, Johnson and Linksvayer, 2010).

We discussed briefly how bees find flowers and what they remember about them in chapter 6 on neurobiology. This is individual-oriented behavior. How scouting is integrated and varies with collective decision making is less well understood. What is clear is that the ratio of scouts to recruits changes with conditions (Seeley, 1983). In times of dearth, there are more scouts than during times of plenty. The mechanism underlying this is not understood, although its adaptive basis seems clear. One hypothesis stresses that lack of activity on the dance floor may trigger a potential recruit to scout, but experimental validation awaits (Beekman et al., 2007).

Individual Preferences and Collective Decision Making

Some important work has explored how the basic algorithm for collective foraging deals with individual-level preferences. We revisit this later, but we review some relevant data here. We explore two questions. First, how do nectar receivers and foraging recruits handle the strong variation in nectar quality and dancing effort on the dance floor, and second, how does preference for nectar and pollen affect collective decision making? For the first question, receivers do not preferentially unload high- over low-quality nectar (reviewed in Seeley, 1995). They do not in effect sample for the best food on the dance floor, but rather unload foragers without reference to the quality of their loads.

Likewise, foragers do not preferentially follow dances for the best sites (Seeley and Towne, 1992). This was shown in an experiment in which the ratio of dances on the dance floor for sites was recorded along with the number of recruits arriving at each location in the field (Seeley and Towne, 1992). The allocation of foragers across sites reflects the ratio of dances for each on the dance floor. In fact, if either receivers or recruits did not behave in the manner discussed here, the basic algorithm would not work nearly so well to allocate bees to sites in accordance with variation in their quality.

The second question is less well understood but equally important. When Seeley and Towne (1992) turned off a feeder being visited by marked foragers and followed the subsequent behavior of the now unemployed foragers, they found that these bees eventually split between scouting and being recruited by dances for new sites. Of those who followed dances, they further split between those following dances for nectar or pollen. This is despite the fact that they had all previously been nectar foragers. Thus, in some sense, the algorithm we reviewed for nectar allocation probably—partially at least—controls allocation to pollen sites as well. We give further reasons why this should be the case in the next section.

Pollen Foraging

There are several differences between pollen and nectar foraging. First, unlike nectar, which is collected to the maximum that the colony is able, pollen is only collected up to a threshold amount (Fewell and Winston, 1992, Camazine, 1993, Pankiw et al., 1998b, Dreller et al., 1999, Calderone and Johnson, 2002). This optimal amount of pollen is dependent on many variables. Chief among these is the number of larvae in the nest (Pankiw et al., 1998b, 2008, Sagili and Pankiw, 2009). Both young and old larvae secrete pheromones (with overlapping and distinct compounds) that trigger increases in pollen foraging. The pheromone secreted by the young larvae, E-beta-ocimene, is the most stimulatory (Traynor et al., 2015). This is perhaps not surprising, as signals in a context like this should precede the need. In other words, a young larva requires little food relative to an older one, so one might think that the young larval signal should be weaker. However, it takes time to collect pollen, particularly when the colony is far short of its needs. Hence, it makes sense that the young larvae should signal their upcoming need so that sufficient pollen is available a few days later when they need it. The amount of empty comb also triggers more pollen collection, although this effect is smaller than that associated with brood (Fewell and Winston, 1992). Finally, adding excess pollen to the nest suppresses pollen foraging (Dreller et al., 1999).

Considerable effort has gone into determining how individual foragers determine the colony state with respect to pollen demand. An early hypothesis that triggered much interest was proposed by Camazine (1993). This self-organizing algorithm proposed an indirect measure of pollen need via the hunger felt by foragers, who were predicted to receive less proteinaceous trophallaxis from nurses when pollen was in low supply. Experimental tests did not support this idea. Studies in which foragers were separated from nurses with screens showed that they could still measure pollen need, and the final test of this idea used a proteinase inhibitor to interfere with digestion to show that forager hunger does not control foraging decisions (Dreller et al., 1999, Vaughan and Calderone, 2002, Sagili et al., 2005, Sagili and Pankiw, 2007). Recent work showing that both brood pheromones are volatile enough to be perceived by all bees in the nest at a distance largely explains earlier work showing that foragers do not need direct contact with brood, or nurses, to regulate pollen collection (Ma et al., 2018). How foragers perceive the amount of pollen and empty comb are still unresolved, but several studies have shown that foragers conduct extensive patrols around the nest as they look for cells in which to unload their pollen (Lindauer, 1952, Dreller et al., 1999, Calderone and Johnson, 2002). Foragers thus have ample opportunity to directly assess the colony state for these variables.

Is the Nectar versus Pollen Forager Dichotomy Real?

The literature gives one the impression that pollen and nectar foraging are separate processes at both the collective and individual bee levels. It is unclear to what extent this is actually the case. First, whether bees collect nectar, pollen, or both depends on the flower. Some plants produce one or the other, some both, and bees are happy to collect both when available (Free, 1993). Second, as Seeley and Towne (1992) showed, unemployed foragers who return to the dance floor after collecting nectar often switch to pollen if the dance they choose to follow is for pollen. There is thus considerable switching at the individual forager level between nectar and pollen foraging. This is particularly true when there are sudden shifts in the demand for pollen (Rotjan et al., 2002). Thus, it may be better to say "forager collecting pollen" rather than "pollen forager" as this stresses the inherent flexibility of foragers to collect what the colony needs, and not necessarily what their individual preferences would dictate.

How Colonies Allocate Foragers to Pollen Patches

Studies have explored whether pollen foragers rate their pollen finds according to the value of the pollen. This seems to build on work using feeders in which bees rank nectar (weight the strength of their dancing) according to sugar content. Pollen sources could vary in the favorability of their amino acid profiles or in their complementarity to recently collected pollen. The data are not terribly supportive of bees ranking pollen in this way, with some studies showing weak support and others no preferences at all (Waddington et al., 1998, Pernal and Currie, 2001, 2002, Cook et al., 2003, Donaldson-Matasci and Dornhaus, 2014, Bixby et al., 2017).

A productive approach to how bees allocate foragers to pollen patches may require a more comprehensive perspective on the allocation to nectar patches. Foraging in the field, as opposed to at a feeder, involves visiting many flowers over a considerable distance (although bees prefer inflorescences and trees that allow them to avoid this problem). It can thus easily be the case that a high sugar concentration is associated with difficulty of collection (individual plants scattered in space, with each offering valuable but small rewards). Weaker sugar content can be associated with copious secretion in a small area. What seems important to the bees therefore is not sugar concentration per se, but rather the net energy gain over a complete foraging trip (Waddington, 1985, Seeley, 1986). This has been shown in experiments in which both distance and sugar concentration are varied and dance responses recorded. A nearby site with dilute sugar water can be more attractive than a more distant site of concentrated sucrose solution (figure 15.2).

A focus on foraging efficiency allows for a straightforward extension of work on collective nectar foraging to collective pollen foraging. Pollen types may not vary in a simple quantitative way, mirroring variation in sugar concentration, but the ease with which pollen can be collected and packed does vary considerably with plant species, as does the distribution over the landscape of the relevant plants. Hence, pollen patches vary strongly in quality, whether they vary in pollen nutritional quality or not, and the model of collective foraging found for nectar should work for collective pollen foraging as well. It is thus not surprising that recruits do not seem to limit themselves to following nectar or pollen dances on the dance floor, but rather are allocated as part of one big

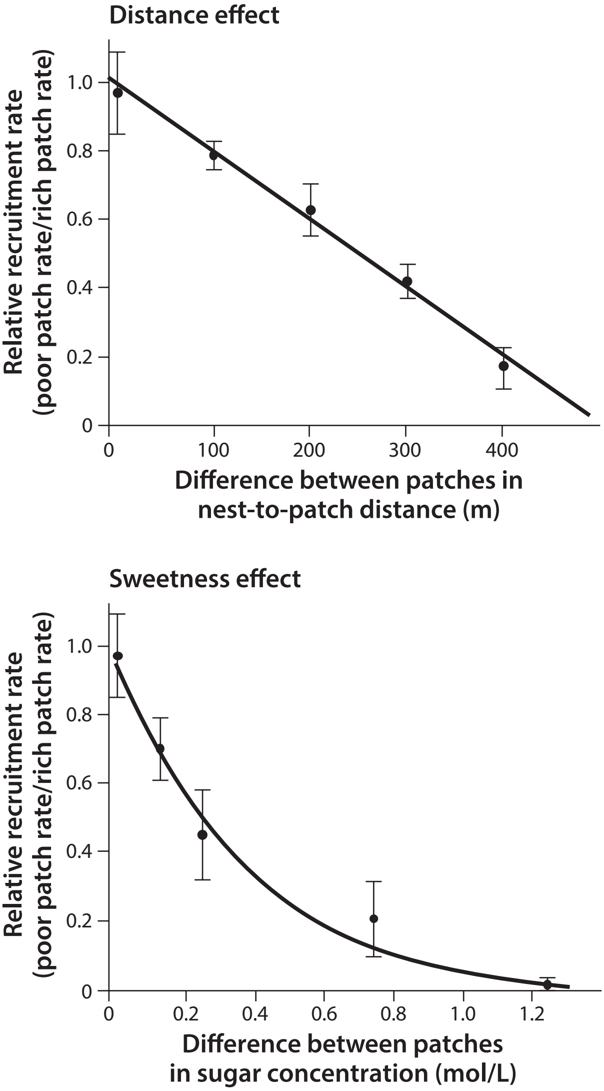


FIGURE 15.2. The distance to a food source and the quality of its reward (in sucrose concentration) affect probability of recruitment via the waggle dance (*from Seeley, 1986*).

system. At the very least, the argument made here should probably be the null hypothesis in studies of how foragers rate pollen patches.

Water Collection

There are always a small number of foragers collecting water to satisfy the colony's need for this vital compound. These bees seem to be specialized for this task, as they show a bias for collection of water over pollen or nectar (Lindauer, 1951, von Frisch, 1967, Calderone and Page, 1992, Pankiw and Page, 1999, 2001). Although water collection is typically low, there are periods when it increases. When the colony suffers heat stress, for example, the background rate of water collection is radically expanded to satisfy the pressing demand for water, which is used to cool the nest via evaporative cooling. Further, when nectar collection is low (as in a dearth) and the colony is subsisting on stored honey, which has a low water content, the need for water also rises. Water needs can also be considerable in the winter, particularly late in the winter when brood rearing begins. At such times, the bees engage in risky trips for water at low temperatures (down to about 4°C).

Water collection can be studied experimentally by shining a heat lamp on the broodnest or heating the room containing an observation hive (Kuhnholz and Seeley, 1997, Johnson, 2002, Ostwald et al., 2016). Experiments that have done so have found that when the demand for water increases, water collectors, who typically have difficulty unloading their water to receivers, are suddenly surrounded by unloaders (Kuhnholz and Seeley, 1997). The number of bees begging for water, and the wait time until unloading, both reliably indicate the colony need for water, and when the indication is that more is needed, the water collectors begin to dance vigorously for their water sources. Recruits are sent to collect water, and the colony's water influx greatly increases. Water foraging stays high until the temperature in the broodnest returns to optimal, at which point unloading water becomes difficult again and water foraging returns to background levels. For all but the most extreme heat stress, the buildup of water foraging does not interfere with other foraging activities, which continue unabated.

Adaptive Basis of the Waggle Dance

The adaptive basis of the waggle dance is a surprisingly poorly understood topic. The origin of this question goes back to Wenner's criticisms of von Frisch's interpretation of the meaning of the waggle dance (Wenner, 1967, Wenner et al., 1969). That question is long resolved, however, as numerous studies have shown, using a variety of clever experiments, that the bees use the dance language largely as von Frisch concluded (Gould, 1974, Michelsen et al., 1989). It is the adaptiveness of the signal at the colony level that has proved difficult to elucidate, although the basic principles are probably now understood.

A series of experiments have made use of the fact that bees dancing on a horizontal surface, who cannot see the sun, produce dances with random angles. Colonies in this state can thus be compared in time and place to control colonies that have functional waggle dances. Sherman and Visscher (2002) showed that in a suburban environment the waggle dance does not increase foraging success for most of the year. Only in the winter when blooming flowers are rare and dispersed did the waggle dance increase foraging success. Dornhaus and Chittka (2004) conducted a similar study across three landscapes: temperate shrubland, temperate agricultural landscape, and tropical forest. They found that the dance was only beneficial in the tropical forest. They pointed out that food sites were more dispersed in that habitat than in the others. Theoretical work has followed, and the current conclusion is that the dance is adaptive when food sources vary strongly in quality and are difficult to find (Beekman and Bin Lew, 2008). The dance is also adaptive when many sites of highly variable quality are available and easy to find, as we reviewed earlier. In this context, the dance allows colonies to allocate their foragers to the highest-quality sites. This effect can be missed in studies that look at foraging rate alone.

Little Evidence for Adaptive Imprecision in the Waggle Dance

A topic with a long history, on which some recent progress has been made, is the question of whether the imprecision in the waggle dance is adaptive. The natural history is quite obvious to anyone observing dances. Although the angle relative to vertical indicates the direction to the destination relative to the position of the sun, the advertised angle varies significantly from run to run. Further, the variation about the central angle varies inversely with the distance to the location being advertised. These facts were noticed by von Frisch and other early authors, and the hypothesis that this is adaptive was proposed (von Frisch, 1967). The idea is that, if the variation in angle decreases with distance to the destination, then the size of the patch being advertised will stay the same as the distance increases (Towne and Gould, 1988). This might be adaptive because food sources are rarely points in space, but rather areas in which the same flowers are in bloom. Hence, sending recruits to a scatter of clustered locations could be adaptive. Weidenmuller and Seeley (1999) collected some evidence to support this idea, as dances for tree cavities during swarming were found to have less variation than food dances. More recent work, however, has largely not supported this idea. The swarm dancing study, for example, was replicated, with the results not supporting different degrees of accuracy in nest site versus food site dancing (Tanner and Visscher, 2006). The discrepancy between studies was attributed to an artifact in the earlier study, as bees were forced to dance on an artificial surface, leading to less error. Further, work was conducted suggesting that the error pattern is an unavoidable physiological consequence of translating the sun-based horizontal information to a vertical signal based on gravity (Tanner and Visscher, 2010a, 2010b). Studies suggested that this problem decreases as the length of the run increases. It was further shown that the variation in dance angle does not change with distance when bees use the sun as the reference, either in *A. florea*, or *A. mellifera* forced to dance with reference to the sun (Tanner and Visscher, 2010a, Preece and Beekman, 2014). Finally, and perhaps most convincing, bees seem to use the average of the dances followed (Tanner and Visscher, 2008). Hence, the variable dances are not used on a one-by-one basis to produce a scatter of advertised sites, but rather averaged by the follower to obtain the best estimate of the direction to a single place, directly contradicting the basic idea of the adaptive hypothesis. In sum, although the question is not entirely understood (Gardner et al., 2007), the data to date better support the notion that the scatter of dances is not adaptive.

Individual-Oriented Studies

Foraging Improves with Experience

The members of the forager caste are quite specialized for their task. We covered the basic physiological nature of this in chapter 12 on division of labor. One aspect of specialization left to review is the nature by which foragers become more efficient with more experience. There are two hypotheses for how this is thought to happen. First, there may be physiological specializations that increase with time. This would mainly involve slimming down in weight (to perhaps carry heavier loads) and changes to flight muscles (Schippers et al., 2006, 2010, Johnson, 2010a). Second, we know that foragers learn to work flower types to maximize their efficiency in collecting either pollen or nectar

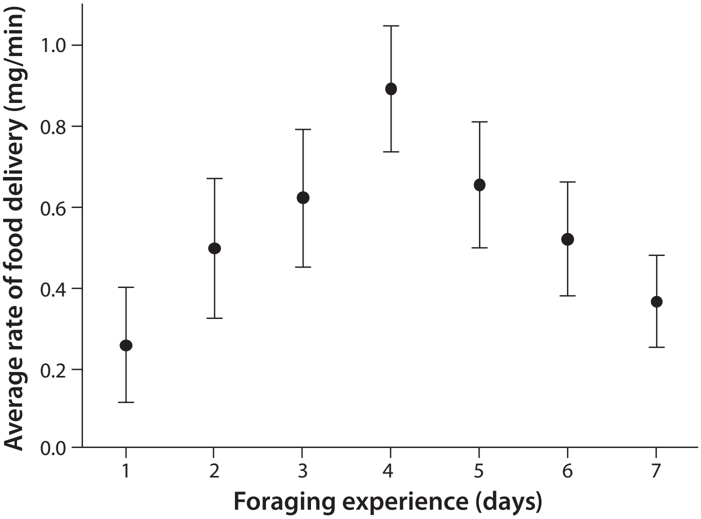


FIGURE 15.3. Forager rate of food delivery as a function of experience when foraging in the wild (*from Dukas, 2008a*).

(Gould, 1991, Dukas and Visscher, 1994, Abramson et al., 1997, Couvillon et al., 2001, Mercer, 2008). Famous examples involve learning to avoid flowers with tripping mechanisms, like alfalfa, and learning to rob flowers (avoid the reproductive parts).

A series of interesting studies by Dukas and collaborators have explored these competing, but not mutually exclusive, hypotheses. This work followed foragers throughout their foraging career to document changes in foraging rate and efficiency. Overall, it demonstrates that bees become two to four times more capable as foragers (measured as net nectar return rate) through the first week of foraging, with a decrease in efficiency following (Dukas and Visscher, 1994, Dukas, 2008b). Whether this increase in foraging ability is due to physiological changes or learning was explored by looking for an increase in foraging ability through time when foraging at a feeder (Dukas, 2008a). A feeder is a simple setup, and learning beyond the first few flights is not expected. Hence, if the increase in foraging ability through time is caused by learning, then we should not see a strong increase when bees are limited to foraging at a feeder. If physiological changes due to flight metabolism and other physiological changes are key, however, then we should see an increase in foraging ability through time at a feeder. The results support a prominent role for learning and little to no role for physiological specialization. Figure 15.3 shows the results from the initial study, showing the increase in net intake when foraging

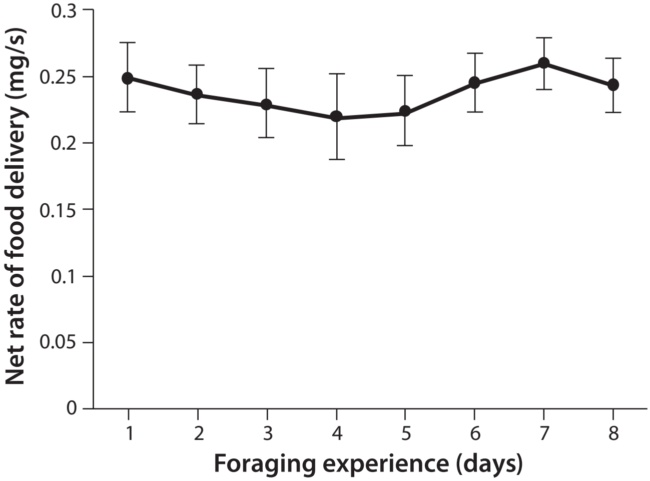


FIGURE 15.4. Forager rate of food delivery as a function of experience when foraging at a feeder (*from Dukas, 2008b*).

naturally, and figure 15.4 shows a lack of increase in efficiency through time when foraging at a feeder.

Response Thresholds

Work on response thresholds largely grows out of studies of artificial selection for pollen hoarding and studies looking for genotypic biases to foraging in general (Scheiner et al., 2004). We reviewed some of this work in chapter 5, where we discussed data showing that pollen hoarding bees have a lower response threshold to sucrose than do wild-type bees or bees selected to collect little pollen (Page et al., 1998, Pankiw and Page, 1999). Page et al. (1998) further showed that pollen foragers within a wild-type colony have lower response thresholds to sucrose than do nectar foragers. Pankiw and Page (2000) then showed that newly emerged bees differ in their sucrose responsiveness, and this variation predicts (to a modest extent) their adult foraging decisions (figure 15.5). This is a key result, suggesting that the variation in response threshold is stable through life and is not entirely dependent on recent experience. It was further found that water foragers have the lowest thresholds, followed by pollen foragers, nectar foragers, bees collecting both pollen and nectar, and bees coming back to the hive empty. Later work showed that foraging experience and feeding within the nest also modulate response thresholds in a dynamic manner

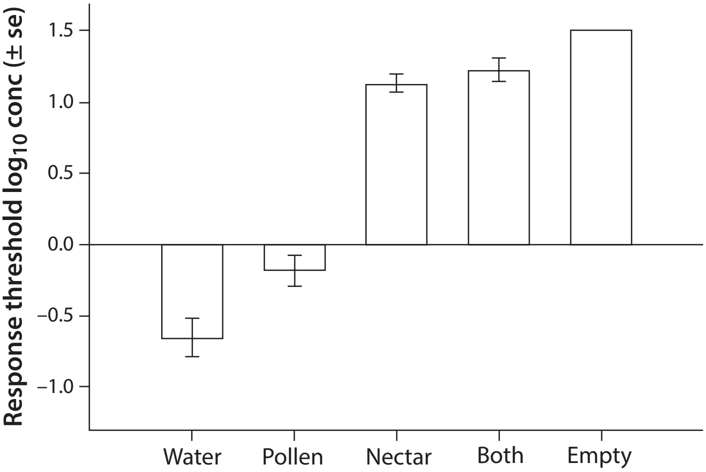


FIGURE 15.5. Response thresholds of foragers returning home with various loads (*from Pankiw and Page, 2000*).

(Pankiw et al., 2001, 2004a). This modulation is in response to many variables, including concentration of nectar encountered in the field or nest, pheromones, and satisfaction of hunger or thirst (Pankiw and Page, 2003).

To date, it is not known if variation in response threshold to sucrose is causative for foraging decisions, but this seems unlikely (Scheiner et al., 2004). Rather, variation in sucrose responsiveness, and all the related traits that go with it, are likely part of a physiological state contributing to biases in foraging decisions. It would be helpful to find some experimental manner of modulating response thresholds without feeding (perhaps pharmacologically) to ultimately determine its effect on foraging decisions.

Integrating Individual-Oriented Datasets with Those Focused on Collective Decision Making

The most pressing problem in the study of honey bee foraging relates to integrating studies at the collective and individual levels. Neither the work of Seeley and others nor that of Page and others makes much reference to the other body of work. This is unfortunate, as experiments designed to bring the two bodies of work together would deepen our understanding of each. We conclude this chapter with a brief discussion of some issues involved and how we might move forward.

The mechanisms controlling foraging must be able to adaptively allocate individuals to forage types and places in the field, and they must allow the colony state to guide this allocation in accordance with current needs. To do this, multiple sources of information must be integrated by diverse members of the colony (Friedman et al., 2020). Although these needs could in principle be accomplished with a simple self-organizing model, in practice they are controlled by sophisticated feedback networks reminiscent of those controlling homeostasis and physiological changes in the body (Seeley, 1995). This is referred to as "social physiology." Such networks likely evolve because they are far more efficient and effective than simpler systems. Feedback loops further work with or without variation in the individuals involved. A colony of identical clones would still be able to allocate foragers to pollen in accordance with variation in brood pheromone, empty space, and pollen stores, for example. Seeley's model for collective nectar foraging likewise works with or without variation in the individuals involved. There are even clonal social insect species that must come close to doing this, although they still have small amounts of developmentally derived variation (Hartmann et al., 2019, 2020). So the key questions with respect to linking the work on individual-level variation and collective decision making regard when individual variation is adaptive and why. In particular, although variation may not be necessary, it may improve some aspects of foraging.

If we consider the work on pollen and nectar foraging relating to response thresholds, how does this variation (bias) fit into the colony-level need to shift large amounts of labor from one to the other? We know bees can do this, as there is a rapid boost of labor devoted to pollen collection when necessary at the colony level and this involves much switching at the individual level from nectar collection (Rotjan et al., 2002). Hence, the key question is how the innate bias for collecting one or the other, which has been clearly shown, interacts with the colony-level needs. Are the mechanistic bases for the response threshold biases modulated by signals from the collective decision-making system? Could the many modulatory signals with poorly understood functions be involved? Does brood pheromone directly modulate response threshold (Ma et al., 2019)? Does the communal stomach hypothesis link both bodies of work? These are all ways in which the colony-level need could be transmitted to foragers in a manner capable of overriding individual-level biases in the proximate mechanisms leading to foraging decisions.

In general, there are many experimentally tractable ways to explore how the dance language, and pheromonal signaling, interact with variation in response thresholds (and other physiological bases for individual variation in behavior). Some clear possibilities were outlined above. Hopefully, future work will tackle these issues. At the very least, it would be preferable for foraging control hypotheses related to variation between individuals (either genetic or experience based) not to be placed in competition with hypotheses elucidating group-level feedback loops, as these mechanisms are not mutually exclusive.

16

Tropical Honey Bees

Tropical Honey Bee Life History

Bees in cold climates must store a great quantity of honey and attain a large population size to survive the winter. Their life history thus involves a period of rapid growth in the spring and early summer, followed by a period of food stockpiling later in the year (reviewed in Seeley, 1985; Johnson, 2010). For tropical honey bees, neither a large population nor vast food stores are necessary, so they essentially skip the second phase of European bee life history. Tropical honey bee life history is therefore focused on rapid population growth. Once an optimal colony size is reached, the colony swarms. Tropical honey bee populations can grow 16-fold in a single year as opposed to 2- to 6-fold in European honey bees (EHBs) (Otis, 1980, Winston, 1991, 1992, Schneider et al., 2004b). This basic difference in life history has consequences for colony- and individual-level morphology, physiology, and behavior, which is the subject of this chapter.

Individual and Population Differences

The emphasis on rapid growth in tropical *Apis mellifera* has many consequences. At the individual level, tropical honey bees are smaller than their temperate zone relatives (Rinderer, 1986, Camazine and Morse, 1988, Winston, 1992). This allows more to be produced with the same resources. They also have eggs that hatch a few hours earlier than those of EHBs (Harbo et al., 1981). Faster development continues through the larval and adult stages. Africanized honey bees (AHBs), for example, complete larval development in about 18.5 days as opposed to 20–21 days for EHBs (Winston, 1979a, 1979b). AHB life span is 12–18 days on average in the summer compared to 15–38 days for EHBs (reviewed in Winston, 1991). Temporal polyethism also differs, with AHBs having a higher fraction of bees in the nursing caste throughout the year (Winston, 1979b, 1994, Winston and Katz, 1981, McNally and Schneider, 1992, 1996). The pattern in EHBs switches from one resembling that of AHBs in the spring (when their life histories are convergent) to one emphasizing a larger fraction of foragers later in the year when food storage is paramount (Sakagami and Fukuda, 1968, Seeley, 2019). At the colony level, EHBs have a large variation in mature population size, but their colonies tend to be at least twice the size of AHB colonies (Winston, 1979b, 1992, Schneider and Blyther, 1988, Schneider and McNally, 1992a, Schneider et al., 2004b).

Nesting and Foraging Differences

Because tropical bees do not need to survive cold winters, they do not require a tree cavity for insulation purposes. Nesting in a cavity is a benefit for colony defense, so it is still preferred, but their preference for cavity nesting is weaker than that of EHBs (Schneider, 1990, McNally and Schneider, 1992, 1996). AHBs will nest under rock faces, on branches, in termite mounds (in Africa), or in urban environments, in semienclosed containers, like car tires (Baum et al., 2011). These differences in nesting preference probably contribute to a larger population density of AHBs relative to EHBs, even within the same habitat.

Due to their emphasis on brood rearing, tropical honey bees collect a higher ratio of pollen relative to nectar than do EHBs, particularly relative to when EHBs are emphasizing honey storage (Rinderer et al., 1984, 1985a, Danka et al., 1986, Schneider, 1989). The nature of the tropical landscape may also cause some differences in foraging. Nectar sources tend to be more spread out (in time and space) in the tropics than in the temperate zone (Winston and Katz, 1982, Schneider, 1989, Schneider and McNally, 1992c). This may affect how the dance language is used, but few formal tests in the native range have been conducted.

Defensive Behavior

Tropical honey bees are generally more defensive than European bees (Collins et al., 1982, Rinderer, 1986, Winston, 1992, Schneider and McNally, 1992a, Breed et al., 2004a). It is difficult to say how much more defensive, as little effort has gone into quantifying how defensive European bees are across their range. Some say that German bees (*Apis mellifera mellifera*), for example, are considerably more defensive than those bees to the south, to the point that many beekeepers will not work with them. It is unlikely that even the most defensive EHBs, however, reach the levels of defensiveness found in most AHBs. It should be pointed out, however, that defensiveness in Africa is variable, as is defensiveness in AHBs. Schneider and Blyther (1988) found that in the Okavango in Botswana, for example, colonies typically flee when their nest is attacked rather than attempt to defend it. The authors attributed this to the low levels of food these bees store and their small colony size, suggesting they cannot tolerate large losses due to stinging. Likewise, Acevedo-Gonzalez et al. (2019) found low (EHB-like) levels of defensiveness in the AHBs on the island of Puerto Rico.

While all western honey bees have the same defensive system, tropical subspecies respond faster and with more determination (Collins et al., 1982, De Jong, 1996, DeGrandi-Hoffman et al., 1998, Breed et al., 2004a). Figure 16.1 shows the results for a representative study on the differences between AHB and EHB defensiveness (Collins et al., 1982). Some recent work has reignited interest in the genetic basis of high levels of defensiveness in AHBs. It has long been suspected that the defensiveness of AHBs is due to hybrid effects, as such defensiveness is not typical in Africa. Early work showed that EHB queens crossed with AHB drones are more defensive than AHB queens crossed with EHB drones (DeGrandi-Hoffman et al., 1998). Other work showed that two gene clusters within a sting quantitative trail locus (QTL) show maternal-biased gene expression (Gibson et al., 2015), suggesting that admixture interaction effects could be pronounced in European and African bee crosses. Harpur et al. (2020) recently looked for single nucleotide polymorphisms (SNPs) associated with defensive behavior within AHB colonies in Brazil. They identified a large number of SNPs associated with defensiveness and also showed that variation in one previously identified QTL, sting 2, showed M-lineage-biased genomic ancestry, again suggesting that interactions between European and African alleles may be associated with the high defensiveness of AHBs. There are other explanatory hypotheses for these phenomena, so future work will hopefully explore this question in more detail. In general, it appears that there are differences between AHBs and the populations they derive from for defensiveness, and interactions between alleles at loci that underlie local adaptation within the original populations is a good hypothesis.

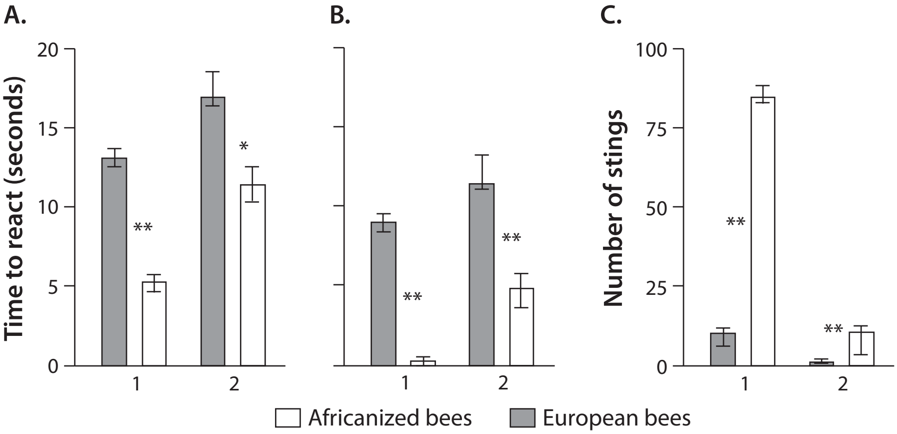


FIGURE 16.1. Defensiveness in AHBs versus EHBs. (A) Time for a colony to react to alarm pheromone at the entrance. (B) Time to react to a moving target swung before the nest. (C) The number of stings on the moving target after 30 seconds (*from Collins et al., 1982*).

Varroa *Resistance*

The mite *Varroa destructor* is an obligate parasite of the tropical *Apis cerana*. In its native host, the mite does not cause colony damage (Peng et al., 1987). This is mainly because the mites cannot breed in worker cells in *A. cerana* (keeping down mite reproduction levels) and because this bee is quite good at removing mites from the nest. In *A. mellifera,* tropical populations are more like *A. cerana* than they are like temperate zone populations (Calderon et al., 2010). Chemical treatments for *Varroa* are accordingly absent across Africa and most of Central and South America (De Jong, 1996). There is some variation, however, with respect to damage from *Varroa,* as the mites seem to cause no damage in Brazil, where the most research has taken place, but slight damage in Mexico (Guzman-Novoa et al., 1999, Vandame et al., 2000, 2002, Medina et al., 2002, Mondragon et al., 2006, Urbina-Romero et al., 2019). It therefore might be beneficial to treat for *Varroa* in some tropical habitats, as it could increase honey yield. Of course, chemical treatments themselves have consequences, so it would need to be determined that the effects of the chemicals are not worse than the effects of the mites.

In general, it is not known why the mites cause minor problems in Mexico but none in Brazil. Initially, it was thought that the mites themselves were the issue, with a less pathogenic species in Brazil (*Varroa jacobsoni*) and a more pathogenic one in Mexico (*Varroa destructor*), but this is not the case, as *Varroa destructor* is now in Brazil and is not causing significant problems (Medina and Martin, 1999, Arechavaleta-Velasco and Guzman-Novoa, 2001, Garrido et al., 2003, Carneiro et al., 2007, Calderon and van Veen, 2008). Some work has tested whether climate, or other ecological factors, could play a role, but these studies show no clear results (Calderon et al., 2010). Less work has explored the idea that differential patterns of hybridization of European and African DNA may contribute to differential genetically determined resistance, although this seems a reasonable hypothesis. With recent genomic sequencing of AHBs and the identification of many relevant SNPs, this could perhaps be pursued in the future.

Much work has focused on why AHBs are generally resistant to *Varroa*. Although some clear causes have been shown, the ultimate reason is not understood. The clear differences relate to grooming and hygienic behavior. Studies in which mites are placed directly on AHBs or EHBs show that AHBs are considerably better (eight times better in one study) at removing the mites from their bodies (Moretto et al., 1991, Moretto, 1997). Moretto (1997) found that AHBs when parasitized react strongly with body movements (probably the grooming invitation signal), which attracts other bees who help remove the mites. Many mites are damaged by the workers' mandibles in this process and presumably disabled and/or killed. This is similar to what was reported in the Asian honey bee, the mite's native host (Peng et al., 1987). Similar studies in which mites are placed into brood cells show comparable results for differences in hygienic behavior. Guerra et al. (2000), for example, conducted a study in Brazil in which mites were directly introduced into closed cells. AHBs removed 59% of infested larvae compared to only 31% in EHB colonies. Similar results were found in Mexico (Vandame et al., 2000, 2002).

Most research on AHB resistance to mites has focused on those effects less easy to experimentally elucidate. In general, mites have lower fertility in AHB colonies than they do in EHB colonies. That is, fewer mites successfully reproduce in AHB larval cells (reviewed in Calderon et al., 2010). This has been shown in studies using AHB or EHB colonies and also in single colonies in which AHBs and EHBs are cross-fostered (Ritter and Dejong, 1984, Camazine, 1986, Medina and Martin, 1999, Calderon et al., 2009). The reason for this has been difficult to demonstrate, as the studies across the range are not always consistent in the effects found (Rosenkranz, 1999, Garrido and Rosenkranz, 2003, Carneiro et al., 2007, Rosenkranz et al., 2010, Calderon et al., 2010). The clearest results show that male mites and the first female mite

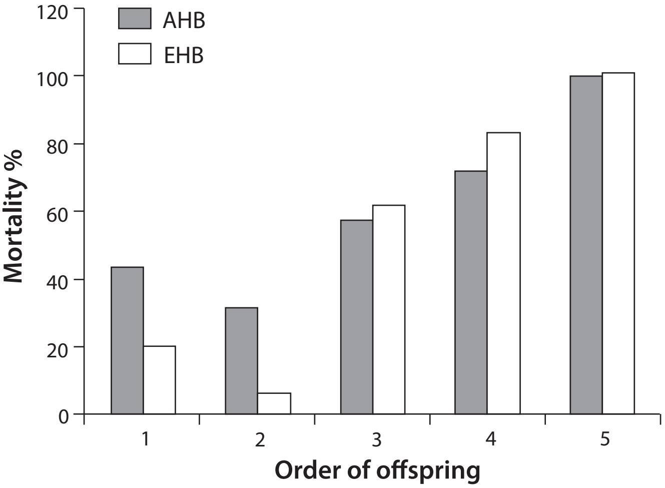


FIGURE 16.2. Mortality of mites by age in AHB and EHB colonies (*from Medina and Martin, 1999*).

(these are the first two eggs laid by the foundress mite) have lower survivorship in AHBs than in EHBs, as shown in figure 16.2 (Medina and Martin, 1999, Calderon et al., 2003, 2009, Martin and Medina, 2004). Given that only a single male is laid per cell, the loss of that male means failure to reproduce. Why male mites die and why mites have lower fertility in general, even controlling for the effect of male loss, have been hypothesized to be due to more movement by the AHB larvae, smaller cell size, or faster development, although none of these has been clearly demonstrated.

The recent work on the survivorship of free-living EHBs by Seeley and coauthors may also be relevant to the survivorship of AHBs parasitized by *Varroa* (Loftus et al., 2016, Seeley, 2017, Seeley, 2019). Frequent swarming and small colony size in EHBs lead to stable coexistence of mites and EHBs. Given that both of these traits are true to an even greater extent for AHBs, they probably contribute to AHB *Varroa* resistance, particularly in the wild.

Absconding and Migratory Behavior

Absconding refers to abandoning the nest and moving elsewhere. Absconding can take place for two reasons. Disturbance-based absconding occurs in response to harassment, or poor nest site, and can occur any time of the year in any honey bee subspecies. Seasonal absconding, better called migratory behavior, is only done by tropical bees. Its mechanistic basis is poorly understood, but given that it is associated with a decline in forage near the nest, its adaptive basis is probably associated with finding nesting sites with better foraging opportunities. In the Okavango, for example, the migratory season corresponds with the period of lowest food availability (Schneider and Blyther, 1988, Schneider, 1990, McNally and Schneider, 1992) and is followed five to six months later by the return of swarms into the area as foraging conditions improve.

Absconding

A temperate zone honey bee colony with tens of kilograms of honey has made a huge investment in their home. If they leave (particularly late in the season), they are almost certain to starve over the winter. Hence, European bees are loath to abscond from their nest. Tropical honey bees have less of a connection to their nest, as they store little food, so it is not surprising that they are much more likely to abscond. Beekeepers working hives is an obvious cause of absconding, but predators or parasites and poor nesting location also reliably cause absconding in African bees (Winston et al., 1979, Hepburn, 1988, Schneider, 1990, Schneider and McNally, 1992b, Neumann and Hepburn, 2011). Needless to say, this makes beekeeping with these bees more difficult than with EHBs.

Migration

Migratory behavior in tropical *A. mellifera* (which also occurs in *A. dorsata*) has many fascinating but poorly understood components. First, preparation for migration begins two to four weeks before departure (Schneider and McNally, 1992b). Food stores and young larvae are eaten, and final departure seems to be associated with the emergence of the last pupa. In the Okavango, only strong colonies migrate (Schneider and McNally, 1992b). Weak colonies try to persist in their current location, and often starve. This seems not to be the case in AHBs in the Americas, as they migrate whether they are weak or strong (Winston et al., 1979, Otis, 1980). During the time the colony is preparing to migrate, the direction of travel, and probably the decision to migrate, are communicated to the colony by migratory dances (Schneider and McNally, 1994a, 1994b). These are waggle dances for long distances (up to 20 km) in which the advertising bee does not make a figure eight (Koeniger and Koeniger, 1980, Lewis and Schneider, 2008). Instead, when the waggle run is produced, the bee runs further in the same direction before doing another run. These dances are quite variable from one run to the next (typically varying from signaling 1 to 10 km), so they are not thought to indicate a particular location but rather a general direction of travel. Consistent with this hypothesis, these dances are performed throughout the nest, not only on the dance floor. This means they are targeted to all castes, not just the foragers. Colonies travel in the direction indicated by the migration dances when they abandon the nest (Schneider and McNally 1994b), but what determines the scouts' decision on the direction of travel is unknown. They may be influenced by the presence of food, or wind direction, but there is little supporting evidence for any hypothesis.

The migration itself is particularly poorly understood. It can be for distances over 100 km from the old nest site and can involve many stopovers in which the bees cluster as they do in swarming (Schneider and McNally, 1994a, 1994b). On the cluster, the bees continue to advertise the direction of travel. Presumably, they also replenish their food stores (fill their honey crops), but this has not been shown. What triggers settling down is also not known. The scouts may engage in a decision-making process during each stopover in which they canvass the area for suitable nest sites and/or forage availability, settling down if conditions are right, otherwise moving on. But this is speculative. Hopefully, future work will continue to explore this fascinating behavior.

The Africanized Honey Bee

Although the most interesting tropical honey bees live in Africa, where there is great variation in behavior and life history due to local adaptation to a multitude of environments, the most widely studied and economically important tropical honey bees are those of the Western Hemisphere, the so-called Africanized bees. These bees are often said to be one of nature's most successful invasive species. This is true, but it is also a window into how people, even scientists, think about EHBs, for which the same can be said, since the European bee is equally successful outside its native range. The EHBs, however, are thought of as being "normal" and good for the environment, while AHBs were initially thought to be bad. The story is changing, however, as AHBs now play the role of EHBs, from an economic perspective, across much of their introduced range and people have begun to wrap their minds around the fact that these bees are here to stay.

The Origin and Spread of AHBs

The origin story of AHBs is quite well known (Otis and Winston, 1981, Winston, 1992, De Jong, 1996, Caron, 2001). EHBs do poorly in the tropics and were never a reliable source of income. The Brazilian government contracted Warrick Kerr to produce a bee adapted to the tropics. He decided to cross African honey bees, which thrive in the tropics but produce little honey, with EHBs to produce a bee with the best characteristics of both. Or at least this is said to have been the plan. In practice, African bees kept in Brazil swarmed, and African queens were sent to beekeepers across Brazil beginning in the mid-1950s, triggering the formation and spread of AHBs.

Initially, it was assumed that the small numbers of African bees would spread via a wave of hybridization such that African drones would mate with European queens to produce hybrid offspring. This was quickly found not to be the case, however. First, the African colonies that spread from Brazil almost completely retained their African behavioral and life history characteristics (Otis, 1980, Schneider and McNally, 1992b, Schneider, 1995, 2015, Rubink et al., 1996, Rangel et al., 2016b). Second, wild populations of AHBs were found to be almost entirely composed of queens with African mtDNA, suggesting that population growth of the wild African colonies was the most important aspect of the spreading process (Sheppard et al., 1991, Segura, 2000, Clarke et al., 2001, 2002, Pinto et al., 2005). We refrain from using the word *feral* to describe AHBs, incidentally, as these bees have been wild for the duration of their history. The notion of their being feral (domesticated animals who escaped) does not fit their biology. They have significant introgression from managed EHBs, but they are essentially invasive wild bees.

Our current notion of how the process of spread works is as follows (Schneider et al., 2004b). Africanized bee populations are thought to build up in the area surrounding the EHBs kept by beekeepers. Soon most of the drones in the area are AHBs, so virgin EHB queens begin to primarily mate with AHB drones, slowly Africanizing many managed colonies. In addition, invasion of EHB colonies by AHB swarms occurs with appreciable frequency, although the process is poorly understood (Danka et al., 1992, Vergara et al., 1993, Schneider et al., 2004a). Through time, EHB mtDNA is lost at the population level, and the resulting bees are entirely African in their maternal lineage. In conjunction with this, a complex process of hybridization occurs within the nuclear DNA that is still poorly understood, as it is variable across the range (Schneider et al., 2004b, Suazo et al., 1998, Suazo and Hall, 2002a, 2002b, Rivera-Marchand et al., 2012, Avalos et al., 2017). It is thought that, due to the selective advantage of African DNA in the tropics, the percentage of European DNA through time decreases to some equilibrium, perhaps reflecting a high proportion of EHB genes affecting neutral traits. What is clear is that the oldest populations of AHBs, those near the site of release in Brazil, are entirely African in their behavior and primarily African in their DNA (Calfee et al., 2020). There are data suggesting that, with respect to introgression, western European bee DNA is more likely to be retained in AHBs (reviewed in Schneider et al., 2004b) and that the combination of a largely African genome with an admixture of western EHB genes conveys survival advantages in the New World (Wallberg et al., 2014). This is surprising since most of the EHBs that the African bees encountered in their spread were eastern European in origin. Finally, there is a hybrid zone extending hundreds of miles in length across both Argentina (and other parts of South America) and California (Calfee et al., 2020). Here African DNA, and African morphology (exemplified by wing length), show a cline of decreasing frequency as one moves into the temperate zone, where EHBs have a competitive advantage. AHBs also did not experience bottlenecks as they spread, as there is no loss of genomic variability as one moves from the point of origin to the temperate zone (Calfee et al., 2020).

Reasons for AHB Superiority in the Tropics

Considerable effort has gone into identifying those traits that make AHBs competitively superior to EHBs in places where they quickly replace them. First and foremost, AHBs have higher growth rates in the tropics (Otis, 1980, Winston et al., 1981, Seeley, 1992a, Rubink et al., 1996). They are better at extracting food from the environment and better able to defend themselves against predators, pathogens, and parasites, and they channel most of their resources into rapid reproduction. These effects are so large that they probably drive most of these bees' ability to replace EHBs in tropical habitats. There are other selective advantages, however. It has been shown, for example, that in hybrid colonies virgin queens with African paternity seem to outcompete virgins with European paternity (Hall, 1999, Taylor, 1999, Schneider and Degrandi-Hoffman, 2003). It is also thought that AHB drones may have a competitive advantage since they are produced in higher numbers and more consistently through the year (at the population and individual colony levels) (Winston, 1979b, Otis, 1980, Rinderer et al., 1987, Quezada-Euaan and

May-Itza, 2001). AHB drones also routinely migrate into EHB colonies, thus reducing EHB drone production (Rinderer et al., 1985b). It is further thought that many African alleles may be dominant. This grows mainly from work on defensive behavior in hybrids, which shows that crosses in which the fathers are African are more defensive (DeGrandi-Hoffman et al., 1998, Guzman-Novoa et al., 2002). In general, all of these things may be true, but it is also possible that even were all these things not true, the spread of AHBs would have been nearly as fast due to the strong differences in basic colony growth rate; essentially, local adaptation to tropical environments.

AHBs and Beekeeping

We end by noting that the initial widespread alarm concerning AHBs was unwarranted. First, AHBs are capable of causing harm and even death, but this is rare now that people know the characteristics of these bees (De Jong, 1996). Beekeepers also keep their AHB apiaries far from human dwellings, which decreases encounters. With respect to economic concerns, the AHBs have been a great success. There was a period of a decade or two in which the beekeepers and scientists were learning how to work with these bees when they were somewhat unproductive, but this is now over and the AHBs are far more economically productive than the EHBs ever were in the tropics (De Jong, 1996). They produce good honey crops and specialty products, such as resin. They are also quite hardy, resistant to *Varroa,* and easily obtained (by capturing swarms) by poor persons across Latin America.

17

Immunity, Parasites, Pests, and Pathogens

Honey bees have two layers of defense: individual, which they share with all insects, and social, which they share with other social insects. We cover both, then switch to reviewing what is known of the diverse array of bacterial, fungal, viral, and arthropod enemies of bees. Colony collapse disorder, along with other larger issues associated with the interactions between colony stressors of many sorts, is left for chapter 19.

Individual-Level Immunity

The Insect Immune System

To understand how an insect defends itself from pathogens in general terms is relatively straightforward. One must understand how the insect recognizes threats, how that recognition leads to the appropriate response (what signal transduction pathways are activated), and what the response is (how the insect kills the threat). We cover one pathogen class at a time, as different systems respond to different classes of threats. This work has mainly been done in *Drosophila melanogaster*, the genetic model system, but, as we will see, the same systems are in place in honey bees.

The insect immune system recognizes microbial threats (bacterial and fungal) by detecting components of their cell wall (Lemaitre and Hoffmann, 2007). These are called pathogen-associated molecular patterns (PAMPs), and they are recognized by pattern recognition receptors (PRRs). PAMPs differ between classes of pathogens and are not present in the insect's body, thus providing a reliable cue that particular invaders are present. The detection of the threat by the PRRs triggers activation of one or several signal transduction pathways, which leads to increased expression of the appropriate response (effector) genes. In the case of microbes, these are often antimicrobial peptides (AMPs). These simple peptides interfere with microbial reproduction, or their metabolism. AMPs come in many forms that target different classes of microbial pathogens. Some cells in insect hemolymph are also activated in response to microbial infection, and these cells engulf the microbes via phagocytosis.

The response to viral threats is different in nature, though it has some conceptual similarity in the beginning phase, as PAMPs and PRRs are involved. When viruses replicate, long, double-stranded RNA structures are formed, and these are not typically present in eukaryotes. These RNA species thus act as PAMPs that activate the RNA interference (RNAi) pathway, which uses the sequence in the double-stranded RNA structure as a template for the breakdown of sequence-specific RNA (e.g., that of the virus). The complete molecular pathway is complex and beyond the scope of this discussion, but RNAi is known to be a potent and specific regulator of pathogen RNA (Belles, 2010). Other pathways not involving RNAi are also involved in viral defense, some specific and some nonspecific with respect to what they target, but as these are poorly understood even in model systems, we omit discussion of them (Merkling and van Rij, 2013).

Insects defend themselves against larger threats by encapsulation of the threat and with the production of reactive oxygen species. This defense is common for insect species that battle large threats, such as parasitoids, but as honey bees do not have major threats in this category, we omit detailed discussion of them.

Honey Bee Immune Studies

GENOME STUDIES

The publication of the honey bee genome showed that bees have a complete RNAi system and functional pathways for all those immune pathways found in the model system (Toll, IMD, Jak/Stat). Perhaps the major result from genomic studies has been to show that bees have relatively fewer immunity-related genes than some other insects (Evans et al., 2006). For example, six AMPs have now been described in bees (apisimin, hymenoptaecin, abaecin, apidaecin, defensin1, defensin2), which is less than the twenty found in *Drosophila* or the nine in mosquitoes (Casteels et al., 1989, 1993). This has been argued to be the result of the specialized social nature of bee life. First, bees feed on food produced by plants in a mutualistic relationship. They do not feed on carrion or other foods likely to be contaminated with harmful microbes (but see the upcoming section on risky foraging). Bees also make use of collective immunity involving the use of propolis, an antimicrobial agent that may make personal investment in AMPs less necessary (Simone-Finstrom and Spivak, 2010).

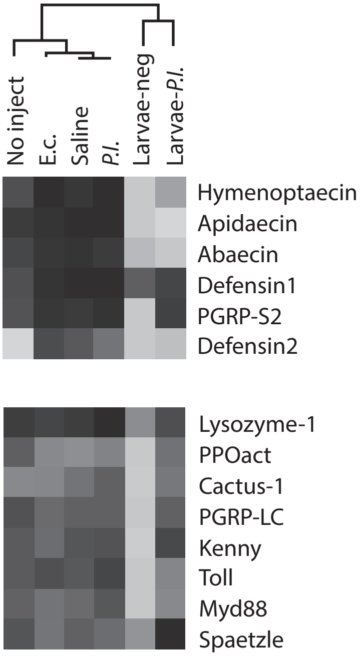


FIGURE 17.1. Immune response (expression of candidate immune genes) of bees challenged with pathogens. Adult bees are shown in the first four columns: a control group and those injected with *E. coli* (E.c.), saline, or *Paenibacillus larvae* (*P.l.*).Bee larvae are shown in the last two columns: a control group and those fed *P.l.* The top panel shows the genes with the strongest response, while the bottom panel shows other genes with weaker but still significant responses (*from Evans et al., 2006*).

INDIVIDUAL IMMUNITY

Honey bee–specific pathogens, along with laboratory microbes, such as *E. coli*, have been injected into bees to show that they have the basic immunity machinery previously reviewed from the model system (Evans and Spivak, 2010). These studies also have the potential to show why these responses fail, or in other words, how the pathogens avoid the response. Injection of *E. coli* or American foulbrood (AFB) spores, for example, leads to the increased expression of several AMPs, as shown in figure 17.1 (Casteels et al., 1989, Casteels-Josson et al., 1994, Evans, 2004, Evans et al., 2006, Laughton et al., 2011). In contrast, when fed the microsporidian parasite *Nosema cerana*, bees show decreased expression of several AMPs, suggesting that this pathogen can suppress the bee immune system (Chaimanee et al., 2012, 2014). In general, it is believed that immune responses to pathogens are somewhat (though not completely) idiosyncratic in nature and unique to each pathogen.

Defense against viruses is complex and still poorly understood, in both the model systems and honey bees (Brutscher et al., 2015, Brutscher and Flenniken, 2015). Injection of viruses, as expected, sometimes does not activate the production of AMPs (Azzami et al., 2012), but it does lead to activation of the RNAi pathway (Maori et al., 2009, Hunter et al., 2010, Chen et al., 2014). Laboratory infection of bees (pupae or young adults) with Israeli acute paralysis virus (IAPV) led to variable phenotypic responses in bees and strong, but complex and difficult-to-interpret, transcriptional responses (Boncristiani et al., 2013, Galbraith et al., 2015). A poorly understood nonspecific antiviral mechanism, not associated with RNAi, has also been shown such that injection of any dsRNA construct (independent of sequence) leads to a decrease in viral pathogen load (Nunes et al., 2013, Flenniken and Andino, 2013). Viral infection also sometimes leads to the activation of pathways typically thought to be associated with defense against microbes, but how this defends against viruses is not understood (Flenniken and Andino, 2013).

RISKY FORAGING

A key question in honey bee immunity is how pathogens get into the hive. Foragers are the only bees who leave the hive for extended periods of time, and they are the most likely suspects for bringing in pathogens (Corby-Harris et al., 2014a). This can occur when foraging, robbing, or drifting (though drifting is probably rare in nature, given the low density of nests). Flowers, for example, harbor viral pathogens and microbes, some harmless and some potentially harmful (Singh et al., 2010, Vannette et al., 2013, Mao et al., 2013, Vannette and Fukami, 2018). Further, Mao et al. (2013) showed that a component of pollen cell walls, p-coumaric acid, causes upregulation of both detoxification genes and AMPs. Likewise, Vannette et al. (2015) showed that the hypopharyngeal and mandibular glands produce massive quantities of AMPs in foragers. Taken together, these studies suggest that foragers are prepared to encounter and respond to a diversity of threats. This contrasts with the traditional notion that the flower, being in a mutualistic relationship with the bee, is a safe place.

NO THROWAWAY CASTE

Amdam and collaborators proposed that foragers are a “throwaway” caste (Amdam et al., 2005a, Seehuus et al., 2006). The basic idea is that because foragers have short longevity it makes little sense to invest in defense, hence

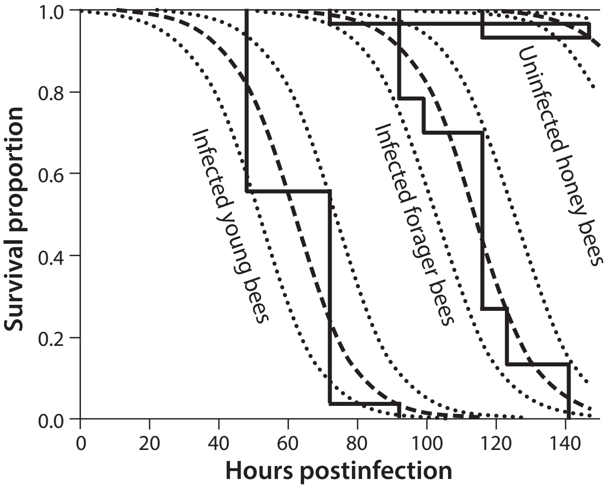


FIGURE 17.2. Survival curves for foragers and young bees infected with pathogenic *Metarhizium anisopliae* (*from Bull et al., 2012*).

their immune systems should be strongly downregulated. It is a good idea in principle, and it became quickly popular after some correlational support for it was collected. Vitellogenin, for example, which is high in young bees and low in foragers, was argued to be an antioxidant (Seehuus et al., 2006). Further, foragers were shown to have lower levels of functional hematocytes and a weaker immune response (measured by AMP expression) to pathogen exposure (Amdam et al., 2005a). Follow-up studies by other authors, however, were not supportive. Wilson-Rich et al. (2008) found no decline in the humoral response in foragers, either in numbers of functional hematocytes or in their ability to encapsulate foreign bodies. Jefferson et al. (2013) found no decline with age with respect to production of AMPs. Most importantly, Bull et al. (2012) showed that foragers survive microbial challenge better than do young bees (figure 17.2). This seems to conflict with earlier results suggesting a weaker immune response by foragers. The problem is that a gene expression response to microbial challenge is a proxy for defense but not a direct test of defensive ability. Foragers, it turns out, have a weaker gene expression response because they already have high titers of AMP proteins. Nurses, who have not invested in making these proteins, must try to catch up when inoculated with microbes by rapidly increasing expression of AMPs. Thus, the forager immune system is prepared for microbial challenge, while the nurses, who do not leave the shelter of the hive, have inactive immune systems at the point of challenge.

Social Immunity

It is fascinating, although maybe expected given the central role sociality plays in honey bee biology, that a honey bee colony can respond to parasites and pathogens as a collective whole (Cremer et al., 2007, Evans and Spivak, 2010). This is an additional layer of immune defense often labeled "social immunity." This topic has seen a surge of interest in recent years, although many of these behaviors have long histories of study.

We briefly cover three mechanisms of social immunity. First, the propolis envelope surrounding the nest and the incorporation of propolis into the wax discussed in chapter 10 is a classic case of social immunity. A second mechanism of social immunity involves a colony-level fever that is induced upon infection (Starks et al., 2000). Essentially, the colony can raise the brood comb temperature, perhaps to suppress microbial growth, although follow-up work has not shown an effect on pathogen load (Goblirsch et al., 2020). A third form of social immunity involves patterns of interaction that protect members of the colony from infection. We give several examples. First, foragers go out and collect food and are exposed to pathogens in the process. It might therefore be adaptive to prevent these workers from interacting with vulnerable colony members. Patterns of food exchange in the nest support this hypothesis, with the young being shielded from interactions with risky partners (Naug, 2008). Second, many studies have now shown that bees change their patterns of interaction when infected with pathogens or parasites. Richard et al. (2008) further showed that activation of the immune system changes the cuticular hydrocarbon (CHC) profile of the bee and also changes its interactions with nestmates. They also showed a transcriptomic link between activation of the immune system and genes in pathways thought to control CHC production (Richard et al., 2012). In support of this work, it has also been shown that bees infested with *Varroa* during development have altered CHC profiles and that bees interact less with bees infected by IAPV (Salvy et al., 2001, Geffre et al., 2020). Third, bees remove both dead and diseased adult bees from the nest. Undertaking is the task associated with removal of the dead adult bees, while hygienic behavior refers to the removal of diseased larvae and pupae (Spivak, 1996, Visscher, 1983). Both tasks are conducted by the same middle-age bees (Arathi et al., 2000, 2006, Perez and Johnson, 2019). The long history of study of this last trait warrants a separate section, which we turn to next.

Hygienic Behavior

Hygienic behavior is perhaps the most important form of social immunity in honey bees. It refers to uncapping the cells of diseased, or parasitized, larvae and removing them, ideally before the disease takes hold (Spivak and Gilliam, 1998a, Leclercq et al., 2017, Spivak and Danka, 2021). That honey bees do this was noticed in the early 1900s during the period of time when AFB was the major threat to beekeeping (Park, 1937). A series of studies demonstrated that the variable resistance to AFB, which was promptly discovered, was at least partly due to resistant colonies removing infected larvae before the bacteria could sporulate (Woodrow and Holst, 1942, Woodrow, 1942). Much later work showed that hygienic behavior is also used to control (partially again) chalkbrood (Spivak and Gilliam, 1993, Invernizzi et al., 2011). The mechanism is similar in that infected larvae are removed before a critical time in fungal development. In general, hygienic behavior, and particularly selection for increased hygienic behavior, is reasonably effective against these pathogens, as shown in figure 17.3 (Spivak and Gilliam, 1998a, Spivak and Reuter, 2001a).

With the arrival of the Varroa mite, much interest was turned toward the study of hygienic behavior with respect to parasitized larvae (Boecking and Drescher, 1991, Boecking et al., 1993). Initial work on *A. cerana* showed that bees control the mite (in part) with aggressive hygienic removal of parasitized larvae, along with removal of mites by grooming (Peng et al., 1987, Boecking and Spivak, 1999). Nevertheless, the primary mechanism of resistance in *A. cerana* is the fact that the mite can only reproduce in drone larvae (Tewarson et al., 1992, Rath, 1999). Parasitized worker larvae quickly die, possibly in a case of social apoptosis (Page et al., 2016, Zhang and Han, 2018). Initial studies in *A. mellifera* also showed strong variation in the rate of hygienic behavior toward parasitized larvae (Boecking and Drescher, 1991), which triggered several long-term breeding studies designed to produce a bee resistant to *Varroa* (Spivak and Gilliam, 1998a, Leclercq et al., 2018b). Work by the Spivak lab, for example, selected for hygienic behavior, largely using freeze-killed brood as a proxy for parasitized larvae (Spivak and Downey, 1998, Spivak and Reuter, 1998, 2001b, Spivak and Danka, 2021). This work was modestly

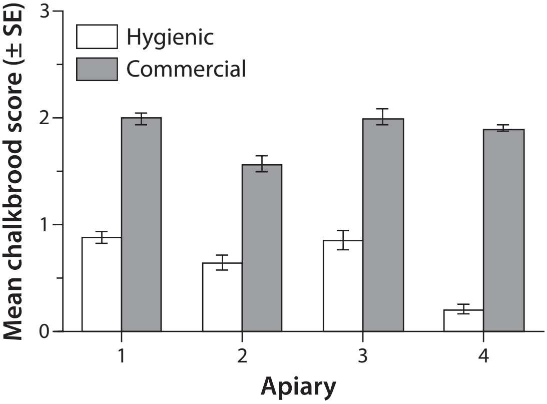


FIGURE 17.3. Severity of chalkbrood infection in hygienic-selected colonies versus nonselected managed bees (*from Spivak and Reuter, 1998*).

successful in producing bees resistant to mites, but not to the extent that the selected lines do not need chemical treatments to survive. A weakness of this work was shown to be a weak correlation between the mechanisms leading to removal of freeze-killed brood versus mite-infested brood (Spivak, 1996, Danka et al., 2013, Leclercq et al., 2018a). This also led to work on the olfactory cues the hygienic bees use (for microbial and parasite infection) and produced behavioral work showing that the middle-age bees are the temporal caste performing this task (Arathi et al., 2000, 2006, Gramacho and Spivak, 2003, Swanson et al., 2009). Recent work has shown that infection with deformed wing virus (DWV), in addition to parasitization by the mites, aids in the identification of larvae for removal (Al Toufailia et al., 2014, Schoning et al., 2012, Wagoner et al., 2018, 2019).

Another major selection effort selected for suppressed mite reproductive success (SMR) and also produced a line of bees with elevated resistance to the mites (Harbo and Hoopingarner, 1997, Harbo and Harris, 1999, 2005). The initial studies found evidence that the larvae changed in response to selection, which was later confirmed (Ibrahim and Spivak, 2006, Harbo and Harris, 2005). However, other work showed that these colonies, subsequently renamed *Varroa*-sensitive hygiene (VSH), also engage in elevated rates of hygienic behavior, likely due to changed larval chemical signaling (Wagoner et al., 2018, 2019).

Honey Bee Parasites and Pathogens

Parasites

TRACHEAL MITES

Tracheal mites, *Acarapis woodi,* are tiny mites that live in the tracheae (breathing tubes) of honey bees. They are a native parasite of *A. mellifera* that was initially discovered about a hundred years ago on the Island of Wight in the UK (Rennie, 1921). These mites are not considered a current threat to beekeeping.

VARROA DESTRUCTOR

Varroa destructor is probably the most important contemporary threat to beekeeping (plate 15) (Spivak, 1996, de Guzman and Rinderer, 1999, Pettis, 2004, Genersch et al., 2010, Martin et al., 2012, vanEngelsdorp et al., 2012, Le Conte et al., 2013, Rinderer et al., 2014, Di Prisco et al., 2016, Decourtye et al., 2019). This mite is native to *A. cerana,* where it does not cause colony losses. The mite initially arrived in the US in 1987, likely having spread from the south, perhaps with Africanized bees who acquired the mites in the 1970s (reviewed in Seeley, 2019). The mite had been present in Europe for a considerably longer time, given that the first introduction of mites to *A. mellifera* seems to have occurred in eastern Russia when Russian beekeepers began keeping western bees in that region (Rosenkranz et al., 2010). Russian beekeepers then spread the mite to eastern Europe in the 1960s from whence it spread west. The arrival of the mites in each region caused a large crash in wild populations, which have since seemed to recover, and an ongoing crisis in managed colonies, which have not.

The life history of *Varroa* is well known. The mites feed on the bee's fat body and have two phases, a phoretic (or dispersal) phase when the mites are on the adult bee and a reproductive phase when the mites are in closed brood cells (Rosenkranz et al., 2010, Ramsey et al., 2019). Mated females disperse on young bees. When nurse bees carrying mites enter a larval cell about to be capped, the mite climbs inside (Boot et al., 1992, Boot, 1992). Mites strongly prefer drone cells, which they can sense via olfactory cues. This is because the longer development time of the drone leads to greater reproductive output (Boot et al., 1995). In Asian honey bees, in fact, the mites can only reproduce on drone pupae, which limits their growth rate. In western honey bees, however, the mites can reproduce on worker pupae as well, although not on queen pupae. The mite lays her first egg, male destined, about 60 hours after the cell is capped. She then lays another egg every 30 hours or so (Sammataro et al., 2000). The eggs hatch and the immature mites feed on a wound in the pupa kept open by the mother mite (Kanbar and Engels, 2003). Brothers and sisters complete development and mate in the cell. The mated females then leave with the newly emerged bee and transfer to a nurse bee to renew the cycle.

The mites have many deleterious effects on bees. It is convenient to discuss these in terms of individual bee effects and colony effects. Bees parasitized by mites emerge lighter in weight than normal and with reduced nutrients in their blood (Rosenkranz et al., 2010). They also have significantly reduced life span (Kovac and Crailsheim, 1988). Further, many bees emerge with viral infections, most importantly DWV, which is vectored by the mite. These bees will not live for more than a few days when the infection is severe and will have greatly diminished life expectancy when infections are less acute (Le Conte et al., 2010). The mites are also known to suppress the immune system of bees, which undoubtedly increases the likelihood of infection by other pathogens and generally weakens the constitution of the bee (reviewed in Rosenkranz et al., 2010).

At the colony level, the mite population grows rapidly and unpredictably (Ifantidis, 1984, Fries et al., 1994, Wilkinson et al., 2001, Wilkinson and Smith, 2002). This is for two reasons. First, the population doubles about once a month due to the intrinsic growth rate of the mites. Second, when a colony dies from massive *Varroa* levels (called parasitic mite syndrome), the mites move, via drifting bees, in massive numbers to neighboring colonies (Shimanuki et al., 1994). Failed colonies are also robbed by neighboring colonies who acquire mites (Peck and Seeley, 2019). The importance of the mite growth rate, of course, is that there are a finite number of mites a colony can support before the drain on the colony leads to rapid death. This number has seemed to vary through time and space, probably due to the pathogenicity of the viruses vectored by the mites and the other stressors on the colony (Hung et al., 1996b, Fries et al., 2006a, Rosenkranz et al., 2010, Natsopoulou et al., 2017).

Controlling the mites in managed colonies is simple in principle, although the methods do not work well at the present time. Essentially, the beekeeper monitors the level of mites in the colony, and when a threshold number is reached, a chemical treatment is given. This knocks back the mite population but does not eliminate it. Because most beekeepers use the same chemicals and because they are incompletely successful, the evolution of resistance to the miticides has been rapid, with mite populations having evolved resistance to several previously useful compounds (Lodesani et al., 1995, 2005, Elzen et al., 1998, Thompson et al., 2002, Elzen, 2003, Elzen and Westervelt, 2004).

RECENT BREAKTHROUGHS IN *VARROA* BIOLOGY

Varroa and DWV. It has been clear for some time that *Varroa* can vector viruses (Bowen-Walker et al., 1999, Martin, 2001, Chen et al., 2004, Shen et al., 2005, Genersch and Aubert, 2010, Di Prisco et al., 2016, Zhao et al., 2019). The recent arrival of mites to the Hawaiian Islands, however, has greatly increased our understanding of how they change the viral landscape of a bee population. Bees were introduced to Hawaii in the mid-1800s. Managed colonies existed alongside feral colonies until 2007 when *Varroa* arrived in Oahu, causing a crash in bee populations. The mite then arrived on the big island of Hawaii in 2009 and was established everywhere within a couple of years. This provided Martin et al. (2012) with the opportunity to study how viral populations respond to the arrival of *Varroa*. In a nutshell, although several viruses were circulating in the population prior to the arrival of *Varroa*, only DWV changed in frequency after the mites arrived. This virus went from low levels (6%–13% of colonies) to very high levels (75%–100% of colonies). Further, a pathogenic strain of DWV became strongly associated with the mites to the exclusion of many other naturally occurring DWV strains. Later work in New Zealand found similar effects, confirming the general importance of these results (Mondet et al., 2014). It thus appears that the presence of mites selects for more pathogenic strains of DWV and increases their ability to infect bees. Later work in the US showed that these dominant strains ultimately diversify, arguably as a means of escaping RNAi by the bee hosts (Ryabov et al., 2014, 2019).

Wild Bees and Varroa. A widespread belief in bee biology has been that wild (in Europe) and feral (in the US) populations of bees were completely wiped out by *Varroa*. This made sense to some given that the bees living in nature are not treated, but it was not consistent with what is known of how novel hosts and pathogens coevolve, which typically involves high initial mortality that falls off as changes evolve in the pathogen and/or the host. Here we cover two series of studies that explored the response of wild or feral bees to the arrival of *Varroa*.

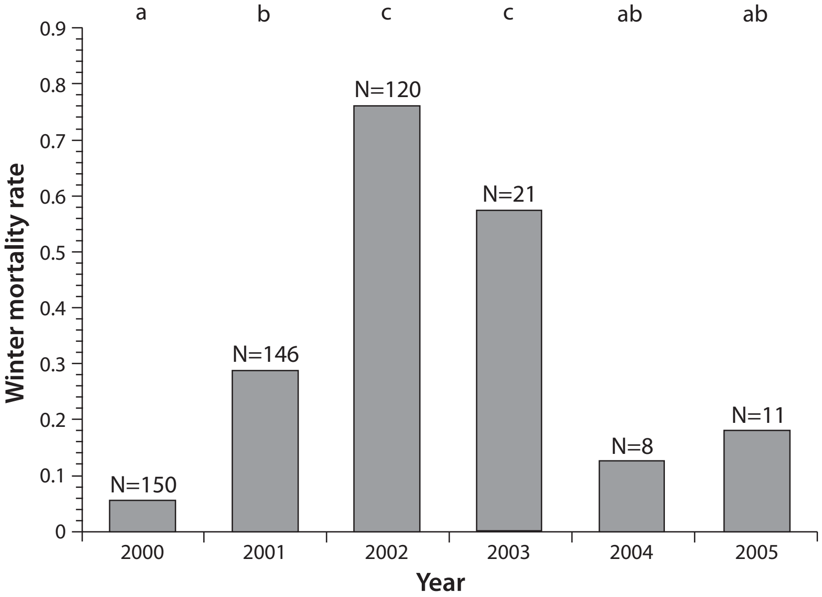


FIGURE 17.4. Mortality in an unmanaged population of honey bees in the presence of *Varroa* on the island of Gotland in Sweden (*from Fries et al., 2006a*).

Studies showed that European bees in California and Arizona fell to very low levels after the arrival of the mites (Kraus and Page, 1995, Loper, 1995). Seeley was interested in whether they had recovered after twenty years (reviewed in Seeley, 2019). He was in an ideal position to answer this question, as he and Kirk Visscher censused a forest for wild honey bee colonies in 1978 (Visscher and Seeley, 1982). He simply had to repeat that study using the same methods. He found the same number of colonies in 2002 as he did in 1978 (Seeley, 2007). He showed that the feral bees in 2002 did have *Varroa* but were apparently not being killed by them (reviewed in Seeley, 2019). Subsequent work showed that the natural habits of nesting in small cavities and frequent swarming prevent mite populations from reaching the toxic levels they do in managed colonies (Loftus et al., 2016). Seeley proposed that beekeepers follow these strategies (allowing hives to swarm and thus stay small) to control *Varroa,* a tactic he named “Darwinian beekeeping.”

Other populations of healthy wild honey bee colonies in the presence of *Varroa* have also been found (DeJong and Soares, 1997, Rinderer et al., 2001, Fries et al., 2006a, Le Conte et al., 2007, Locke, 2016). A population on the Swedish island of Gotland has received perhaps the most attention. Here, 150

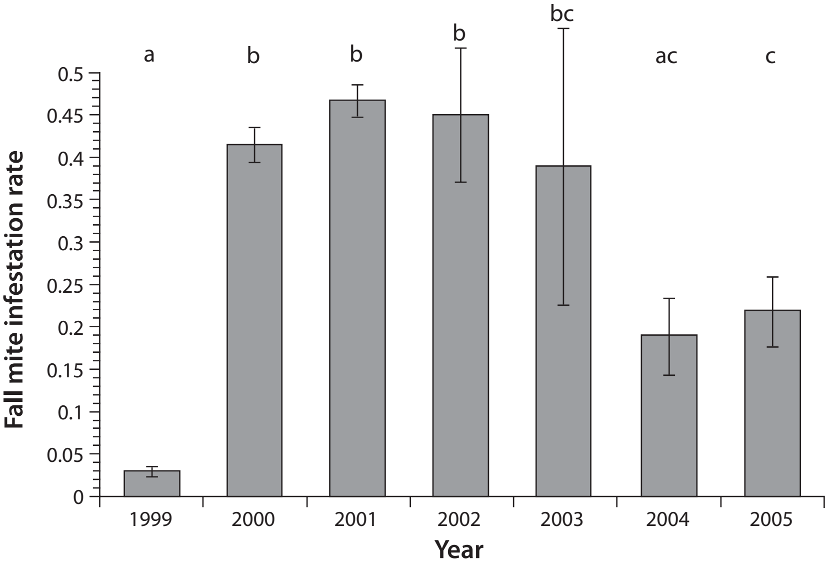


FIGURE 17.5. Mite infestation rates of an unmanaged population of honey bees in the presence of *Varroa* on the island of Gotland (*from Fries et al., 2006a*).

colonies were established and inoculated with equal numbers of mites in 2000 (Fries et al., 2003, 2006b). Figure 17.4 shows mortality levels in these colonies through time. As expected, early on (for two years) the colonies suffered very high mortality. However, by year four winter mortality had fallen to typical levels and the number of mites on bees had dropped by about one-half (figure 17.5). Many studies have now followed up on this initial work to explore changes in the bees, mites, and viruses. Fries and Bommarco (2007), for example, in common garden experiments (resistant and susceptible colonies in the same apiaries inoculated with mites from one or the other population) showed that the bees themselves have evolved resistance, as mites from both sources had significantly lower growth rates on mite-resistant (MR) colonies versus mite-susceptible (MS) colonies. Locke and Fries (2011) then showed that MR colonies are smaller than MS colonies and rear much less drone brood. They did not find a difference in hygienic behavior. Behrens et al. (2011) conducted a QTL study using crosses between MS and MR bees and identified three QTLs for mite resistance. Lattorff et al. (2015) then found evidence for recent positive selection near these QTLs and identified candidate genes within some of them. Locke et al. (2014) showed that the MR bees have high DWV levels but seem to be more resistant to their effects than are MS bees. Later work has largely supported this, although the issue is poorly understood (Thaduri et al., 2018, 2019). Finally, Oddie et al. (2018) showed that rates of capping and uncapping closed brood cells, which interferes with mite reproduction, are higher in MR colonies than they are in MS colonies.

Colony Pests

WAX MOTH

The greater wax moth (*Galleria mellonella*) and the lesser wax moth (*Achroia grisella*) are old and well-known pests of honey bees (Ellis et al., 2013). The female moth lays her eggs on comb and the larvae consume the wax, pollen, and honey. In nature, these pests are mainly a threat to weak colonies, as strong colonies can kill the larvae. The moths are thus more like scavengers of dead or dying hives than they are pests. The main issue with these pests, of course, is that beekeepers store large numbers of wax combs, and if they are not stored properly, the moths can grow exponentially in them and destroy this important resource. Airtight storage of frames, or the use of mothballs, is typically used to protect against this pest.

SMALL HIVE BEETLE

The small hive beetle (*Aethina tumida*) is an emerging threat to beekeeping outside its natural range in sub-Saharan Africa (Ellis and Delaplane, 2008). This beetle seems to do best in hot areas, but it has spread into the temperate zone in North America and Europe (Ellis and Hepburn, 2006). The beetle is also found in Australia and South America (Al Toufailia et al., 2017). Its life history is quite interesting. Beetles pupate in the soil and move into the nest as adults (Ellis et al., 2004). The females lay masses of eggs in crevices or other areas of the hive inaccessible to bees. Like the wax moth, the beetle larvae are happy to consume everything in the nest if undefended. The mature larva leaves the hive and enters the soil to pupate. The beetles are capable of multiple generations per year. Like the wax moth, the hive beetle is mainly a threat to weak or dying colonies. Unlike the moth, however, and more like *Varroa,* the small hive beetle has been shown to potentially vector pathogens (Cilia et al., 2018, Nanetti et al., 2021). Schafer et al. (2010) showed that the beetles can vector American foulbrood (AFB), while Eyer et al. (2009) showed that the beetle can carry bee viruses and potentially cause their spread. How significant a vector the beetle is in the field is unknown but is worthy of more study. There is also some indication that this pest behaves differently in the introduced range relative to the native range, and that European bees respond differently to it relative to African bees, making it a worse threat in the west (Ellis and Delaplane, 2008, Cuthbertson et al., 2013). Finally, bees, particularly those in the native range, often respond to low levels of infestation by imprisoning the beetles into the cracks and crevices in which they hide with propolis (Neumann et al., 2001, Ellis et al., 2003).

Pathogens

There are a great many known pathogens of bees. Most are fungal, bacterial, or viral in nature, but there may be important trypanosomes and other protistan pathogens as well (Schluns et al., 2010, Schwarz and Evans, 2013, Runckel et al., 2014, Schwarz et al., 2015, Ravoet et al., 2015b). Ravoet et al. (2013), for example, found that levels of infection by trypanosomes are predictive of winter mortality. In general, many bee diseases are specific to the brood or adults, although recent work suggests some that were previously thought to be life stage specific may affect all life history stages (Eiri et al., 2015). Here we provide an overview of the major classes of pathogens known to severely affect honey bees.

BACTERIAL DISEASES

American Foulbrood. American foulbrood is a disease of bee larvae caused by the bacterium *Paenibacillus larvae* (White, 1921). Prior to the arrival of *Varroa*, this was the most destructive enemy of the honey bee in commercial operations (Hansen and Brodsgaard, 1999). The biology of this pathogen is well known, and a large body of work exists from the older literature, reviewed by Genersch (2010a). The spores of the bacteria are passed from hive to hive primarily via robbing and drifting bees. Larvae less than three days old are infected by consuming spores. Older larvae are immune to the spores and drone larvae seem less susceptible than worker or queen larvae. The bacteria consume the larvae, which dissolve into a gooey mass. The classic test for this disease is to stick a toothpick into cells containing dead, infected larvae and pull, as this produces a ropy goo. The dead larva eventually dries into a scale that sticks to the wax. Infections can be severe and lead to colony failure. The main honey bee defense is hygienic behavior (Spivak and Danka, 2021). As spores are immortal, what to do with infected colonies, and their equipment, has always been an issue. The classic approach was to burn all equipment, while irradiation is a modern alternative. Treatment is as for all bacteria diseases: antibiotics. The primary antibiotic for AFB, oxytetracycline, has been effective in the US since the 1950s. Perhaps 25% of samples now show evidence of resistance to the antibiotic, however. Newer antibiotics, including tylosin, remain effective based on annual diagnostics by the USDA-ARS Bee Disease Diagnostic Service. Overall, this disease does not appear to be a major contemporary threat to beekeeping; however, given its pathogenicity, it must be closely monitored (Forsgren et al., 2018).

European Foulbrood. European foulbrood (EFB) is a disease superficially like AFB but caused by the bacterium *Melissococcus plutonius* (Bailey, 1956). As for AFB, there is a large body of work from the older literature (Forsgren, 2010). The key differences are that EFB is not as severe as AFB, as a strong colony can usually control the infection, and the resulting dead larvae differ in character. Larvae succumbing to EFB produce scales that are loose in the cell and not infective, unlike those of AFB. In general, EFB is passed from nurses to larvae less than two days old during feeding. The bacteria break through the larval gut wall and typically cause death within a handful of days. Before death, however, infected larvae can be identified by nurses and removed, which may be what allows healthy colonies to control the infection (reviewed in Forsgren, 2010). The primary effect of EFB on the colony is to slow population growth, which both limits honey production and decreases the likelihood of successful overwintering. As for AFB, EFB can be treated with antibiotics (Thompson and Brown, 2001).

FUNGAL PATHOGENS

Chalkbrood. Chalkbrood is a common honey bee disease caused by the fungus *Ascosphaera apis* (Spiltoir, 1955). The fungal spores infect three- to four-day-old larvae after they are consumed (Aronstein and Murray, 2010). The fungus quickly consumes the larva, leaving behind a “chalk mummy.” Chalkbrood is a chronic infection of honey bee colonies that appears to get worse as colonies weaken, perhaps because weak colonies are unable to properly thermoregulate. A healthy colony quickly removes chalk mummies, whose appearance on the bottom board of the hive is quite noticeable. As for other brood diseases, hygienic behavior is the main honey bee defense against this pathogen (Spivak and Danka, 2021).

Nosema. Nosema disease is caused by the microsporidians *Nosema apis* and *Nosema ceranae* (Bailey, 1955, Fries, 1993, 2010, Higes et al., 2006, Chen et al., 2008, Alaux et al., 2010a, Williams et al., 2011, Chaimanee et al., 2012, Rangel et al., 2016a). A third species was recently discovered in Africa but has not yet been the focus of much study (Chemurot et al., 2017). All forms of the disease are spread via spores ingested by bees (in all castes) and involve severe infections of the midgut epithelium (Fries, 1993, 2010, Holt et al., 2013, Milbrath et al., 2015). *Nosema apis* is native to the western honey bee. This species causes a dysentery-like illness. *Nosema ceranae* is native to the eastern honey bee and, like *Varroa,* is more pathogenic in western honey bees than in its native host (Paxton et al., 2007). In western honey bees, *N. ceranae* causes a chronic illness associated with a weakened immune system and decreased life span (reviewed in Fries, 2010). *N. apis* has some seasonality to its prevalence in the hive, while *N. ceranae* was initially more unpredictable but may be becoming seasonal as well (Martin-Hernandez et al., 2007). Coinfections occur and may be worse than infection with one species alone (Milbrath et al., 2015). Finally, it appears that *N. ceranae* may have outcompeted and replaced *N. apis* in the United States, and likely many other places (Rangel et al., 2016a).

Much attention was focused on *N. ceranae* during the colony collapse disorder (CCD) crisis, as work out of Spain suggested that this illness may cause CCD (Higes et al., 2008, 2009, 2013). Studies elsewhere in the world, however, did not find a link between Nosema and CCD (vanEngelsdorp et al., 2009b, Gisder et al., 2010, Martin et al., 2013). Nevertheless, *N. ceranae* is a significant problem for commercial beekeepers, particularly as the treatment for *N. apis,* which appeared to control that pathogen, does not work for *N. ceranae* (Williams et al., 2011). There is also some evidence that antibiotic treatment in general, sometimes used to control bacterial diseases, may actually increase the prevalence of *N. ceranae* by disrupting the normal bee microbiota (Li et al., 2017). At present there is no effective commercial treatment for *N. ceranae.*

VIRAL DISEASES

Over twenty honey bee–associated viruses have been identified (Allen and Ball, 1996, Chen and Siede, 2007, Genersch and Aubert, 2010, Grozinger and Flenniken, 2019). Historically, about seven viruses were diagnosed based on bees suffering from easily noticeable infections (reviewed in Allen and Ball, 1996). The rest of the viruses have been found in deep sequencing efforts, and their effects on the bees are unknown (McMenamin and Genersch, 2015). An important point about viral diseases is that none are currently treatable. Honey bee viruses can be spread vertically or horizontally, depending on the virus and the presence or absence of a vector (Chen et al., 2006, Chen and Siede, 2007).

Deformed Wing Virus (DWV). DWV is far and away the worst honey bee viral disease (Lanzi et al., 2006, Highfield et al., 2009, de Miranda and Fries, 2008, de Miranda and Genersch, 2010, Martin et al., 2012, Schroeder and Martin, 2012, Francis et al., 2013, Nazzi et al., 2012, Amiri et al., 2018). DWV infects all life history stages, but the effects are most apparent in newly emerged adults, who have shrunken wings and bodies. It appears that levels of infection vary from slight, with no obvious effects, to severe in a dose-dependent manner (Shen et al., 2005, Tentcheva et al., 2006). As we discussed earlier, this virus is closely associated with *Varroa* (Gisder et al., 2009, Martin et al., 2012). In particular, mites with the highest viral levels (the virus replicates in the mite) are capable of inducing the full DWV condition (Ongus et al., 2004, Yue and Genersch, 2005, Gisder et al., 2009). In colonies suffering from parasitic mite syndrome, the number of bees with severe DWV infections is high and bees with shriveled wings are commonly observed on combs. It is likely the case that the natural transmission of DWV involves a route through the gut, which provides the bee many opportunities for defense. Vectoring by the mite may bypass these natural defenses, greatly increasing the virulence of this pathogen (reviewed in de Miranda and Genersch, 2010). Evolution for spread via a vector likely also selects for increased pathogenicity, as it improves the mobility of the virus (Brettell and Martin, 2017).

Acute Bee Paralysis Virus (ABPV), Chronic Bee Paralysis Virus (CBPV), and Kashmir Bee Virus (KBV). ABPV, KBV, and CBPV are different but related viruses that cause similar diseases in bees involving trembling and shiny black thoraxes (Blanchard et al., 2007, Celle et al., 2008, Ribiere et al., 2010, Bailey, 1965). ABPV is more virulent, though adult bees can test positive for all of these viruses and show no obvious effects (Hung et al., 1996a). None of these viruses are thought to be economically important, although all are worldwide in distribution. ABPV and KBV can be vectored by mites (Chen et al., 2004, Shen et al., 2005), although neither is strongly associated with them in the manner of DWV.

Israeli Acute Paralysis Virus (IAPV). IAPV was first identified in Israel in 1991 and was shown to cause symptoms similar to ABPV (Maori et al., 2007). There is sequence similarity between this virus and ABPV (and KBV) as well (de Miranda et al., 2004). The virus came to public attention when a metagenomics study identified it as a potential cause of CCD (Cox-Foster et al., 2007). Subsequent studies did not confirm an association (vanEngelsdorp et al., 2009b). This virus was also used in a proof-of-concept study designed to show that RNAi could control honey bee viruses (Hunter et al., 2010). Finally, a recent study suggests that IAPV may change cuticular hydrocarbon profiles such that infected bees may be more likely to be accepted into foreign nests, perhaps increasing transmission (Geffre et al., 2020).

Sacbrood Virus (SBV). Sacbrood virus was one of the first honey bee viruses discovered (White, 1913). It affects all life history stages but is more virulent in larvae. It leads to larval death in the form of a fluid-filled sac. The fluid is full of viral particles, which likely contaminate adult bees as they remove the dead larva from the cell. Larvae are infected by nurses as they are fed (Bailey, 1982).

Black Queen Cell Virus (BQCV). BQCV affects queen and worker larvae and pupae. It was first identified based on the darkening of the queen cup walls that occurs as the disease progresses to completion (Bailey and Woods, 1977). In general, infected larvae have an appearance like that of those infected with sacbrood virus; that is, a toughened cuticle filled with fluid. Some older work suggests an interaction between BQCV and Nosema infection (*N. apis*), leading to increased mortality in bees doubly infected (Bailey, 1982), but this has not been followed up on in recent years. This is important because the major Nosema pathogen is now *N. ceranae*, which has significantly different biology than *N. apis*.

Other Viruses. CCD triggered a massive search for novel honey bee pathogens (Cox-Foster et al., 2007, Runckel et al., 2011, Granberg et al., 2015, Remnant et al., 2017, McMenamin and Flenniken, 2018). This work identified many new viruses, and other pathogens. To date, these are perhaps best thought of as candidate viruses that could be honey bee pathogens, as it is difficult to control for contamination in such studies. Experimental studies with the viruses showing that they can infect bees, and their effects, are necessary to go from candidate pathogen to known pathogen. Nevertheless, the large number of viruses, trypanosomes, and other pathogens present in metagenomics datasets provides an invaluable starting point for future studies.

Pollinator Viruses, Not Bee Viruses

An important question regarding honey bee viruses and other pathogens is, To what extent are they really honey bee pathogens? Are these viruses, for example, honey bee specific, or are they common to many pollinators or even insects in general (Celle et al., 2008)? Further, can flowers be sites of transmission? Foragers of many species, after all, visit flowers, and they would be ideal sites for pathogens to lie in wait. Singh et al. (2010) showed that pollen contains important viruses, such as DWV and sacbrood virus. They further found many of these same viruses in other pollinators (bumble bees, solitary bees, and even wasps). Finally, they found a correlation between proximity to apiaries and rates of infection for IAPV in non-*Apis* pollinators. While the study described only the presence of viral particles and not pathogenicity, this work suggests that some "honey bee viruses" are actually "pollinator viruses," and there is an interesting community ecology component to their epidemiology. Further, it suggests a powerful way that managed honey bee colonies may negatively affect wild pollinator communities (Graystock et al., 2016). Much contemporary work has followed up on this study (Levitt et al., 2013, Ravoet et al., 2014, Furst et al., 2014, Dolezal et al., 2016b, Martin and Brettell, 2019).

It should be stressed, with respect to pathogen spillover, that most studies to date have simply shown that other pollinators encounter honey bee pathogens and have not tested the ability of the viruses (or other pathogens) to actually infect other bees. Gusachenko et al. (2020), for example, found that bumbles bees can be infected by DWV when injected (an unnatural transmission route), but are unlikely to be infected when the particles are consumed (the natural route of potential transmission). In general, a pathogen-by-pathogen series of studies is necessary to determine which of the honey bee pathogens are both encountered by other bees and actually infecting them. Finally, and perhaps most importantly, as Jaime Ellis pointed out (personal communication), it is unclear what direction the transmission is going. In some cases, it may be that honey bees are picking up other bee pathogens.

RNAi: The Treatment of the Future?

RNAi has the potential to treat pathogen infections in bees without the myriad off-target effects common in other approaches (Brutscher et al., 2015, 2016, 2017). For this reason, it is probably the approach with the most promise for long-term, environmentally healthy solutions to the many pathogenic issues facing bees. Work on this important topic has taken off within the last few years. Thus far, RNAi studies have been conducted on the treatment of bees for viruses, *Nosema*, and *Varroa*. We discuss each in turn.

Maori et al. (2009) showed that RNAi can suppress IAPV infection in bees in the lab. A field trial involving 160 colonies also showed some promise, in that colonies treated with both the RNAi construct plus IAPV did better than those receiving the virus alone (Hunter et al., 2010). With respect to work on controlling *Nosema*, two studies have fed dsRNA to bees, targeting various key *Nosema* genes with some success. He et al. (2021) targeted spore wall proteins, while Kim et al. (2020) targeted genes in the mitosome (the *Nosema* version of the mitochondria). Both studies showed that feeding dsRNA caused decreased spore counts and increased bee survivorship. Li et al. (2016) targeted the naked cuticle gene (*nkd*), which they showed is upregulated by *Nosema* in order to suppress the bee's immune system. Knockout of *nkd* with RNAi (ingested by the bees) increased bee AMP production, decreased *Nosema* spore counts, and increased bee survivorship. With respect to *Varroa*, early work showed that targeting of key *Varroa* genes with RNAi can cause mite mortality (Garbian et al., 2012). The most exciting study was recently published showing that genetic engineering of an obligate honey bee microsymbiont, *Snodgrassella alvi*, causing it to produce dsRNA targeting either DWV or *Varroa*, causes either suppression of the virus (and increased bee survivorship when infected with DWV) or elevated mite mortality (Leonard et al., 2020). The symbiont also delivers the dsRNA product to the whole bee body, allowing for RNAi in any tissue—something not possible before. Although the cost of producing dsRNA, and its fragility in the environment, make real-world extension of the approach difficult, this work has the potential to ultimately control many pathogens and parasites.

18

Detoxification and Pesticides

Prior to the large-scale introduction of systemic pesticides, the compounds used for pest control in agriculture were quite toxic to bees and would typically kill on contact (Mussen, 2015). The problem for bees was thus one of avoiding the pesticides, which is relatively easy given the mobility of hives. Beekeepers coordinated with farmers to avoid having their bees near the field during sprays. Accidents happened, but they were not considered a major threat to commercial beekeeping. With respect to fungicides and herbicides, which have always been encountered by foraging bees, the reason for little concern in the past was similar. Although we now know that these compounds, which do not directly harm bees, can interact with insecticides to cause damage, pesticides were usually not in the hive to allow for the interactions.

It is clear that the paradigm around bees and pesticides changed with the advent of systemic pesticides and with the rise in the use of miticides to control *Varroa* (R.M. Johnson et al., 2010). Neonicotinoid pesticides remain in the environment for long periods of time, and bees face a chronic stress from them (Blacquiere et al., 2012, van der Sluijs et al., 2013). Miticides, of course, are directly applied to hives—sometimes at high levels—and may remain in the comb in perpetuity (Mullin et al., 2010, Traynor et al., 2016). Many have argued that these new pesticide stresses have contributed to the large-scale losses now plaguing the beekeeping industry, and a surge of interest in the study of the effects of pesticides on bees has been the result.

Recent work on pesticides and bees has advanced along two main fronts, which provide the structure for this chapter. First, the study of basic detoxification mechanisms has grown considerably (Berenbaum and Johnson, 2015). Many fascinating discoveries have been made with respect to both natural xenobiotics and pesticides. Second, a large body of work has now looked for negative health effects on bees in the field and in the laboratory (Blacquiere et al., 2012, Lundin et al., 2015). These studies have explored the sublethal effects of pesticides in many biological contexts. Tying these effects to real-world losses, however, has proved difficult, as field studies are a mixed bag, with some suggesting significant effects of pesticides on colony survivorship and others little to no effect (Goulson, 2013, Sanchez-Bayo et al., 2016, Schmuck and Lewis, 2016). A particular source of difficulty is getting a true control group, as pesticides are routinely used in all the relevant commercial environments. The question as to whether pesticides alone commonly cause large-scale colony mortality is thus unresolved; currently, the most commonly expressed conclusion in the literature is that pesticides (in all classes) are major stressors that interact with many other stressors to contribute to colony losses (Maini et al., 2010, Genersch, 2010b, Williams et al., 2010, Cornman et al., 2012, Smith et al., 2013, Seitz et al., 2016, Brandt et al., 2016).

Detoxification in Honey Bees

All insects make use of the same basic molecular system for defense against xenobiotic compounds (Heckel, 2014). Generally speaking, the process involves deactivating the toxic substance and converting it into a form that can be excreted from the body. This involves a sequence of molecular responses that can be broken up into three phases (Berenbaum and Johnson, 2015). In phase I, enzymes metabolize the compounds, making them less able to harm the organism. This activity is mainly done by genes in two families, cytochrome p450s and carboxylesterases (CCEs), although genes in other families can also be involved (Sehlmeyer et al., 2010). Phase II involves the addition of glutathione to the substances resulting from phase I, which is necessary if they have not been made sufficiently hydrophilic for excretion (Ahn et al., 2012). This action makes the compounds more water soluble and ready for excretion. This action is performed by glutathione-S-transferases (GSTs). In some cases, GSTs can act on the toxic compounds directly, without action from phase I genes. Finally, phase III involves movement of the conjugated products out of the cell for excretion. This is done by transmembrane proteins in the ATP-binding cassette family, with multidrug resistance proteins being major players (Dermauw and Van Leeuwen, 2014).

The sequencing of the honey bee genome showed that bees have functional members of genes in all three detoxification phases. It was immediately apparent, however, that bees had few of these genes relative to other sequenced insects (reviewed in Berenbaum and Johnson, 2015). Initially, it was unclear

Table 18.1. Detoxification gene family sizes for the honey bee and other insects

	Apis mellifera	*Bombus huntii*	*Megachile rotundata*	*Nasonia vitripennis*	*Drosophila melanogaster*
p450	46	44	52	92	85
CCE	24	23	22	41	35
GST	10	11	9	16	38
UDP-GT	12	2	2	22	34
Total	92	80	85	171	192
Life history	Advanced eusocial	Primitive eusocial	Solitary bee	Solitary parasitoid	Solitary saprotroph
Order	Hymenoptera	Hymenoptera	Hymenoptera	Hymenoptera	Diptera

Source: Berenbaum and Johnson, 2015.

if this was a honey bee–specific trait, a bee trait, or something common to the Hymenoptera. Later work demonstrated that honey bees have a typical detoxification system for bees and that bees in general are less gene rich in this context than other Hymenoptera and most other insects (table 18.1).

The low number of detoxification genes in bees was initially thought to cause bees to be particularly sensitive to pesticides (Claudianos et al., 2006), but later work demonstrated that when challenged with pesticides one at a time bees are no more sensitive than other insects (Hardstone and Scott, 2010, Arena and Sgolastra, 2014). A currently popular hypothesis, still based on the small size of bee detoxification gene families, is that bees should be more susceptible to combinations of pesticides. Although data now show that bees are sensitive to combinations of pesticides (reviewed later), it has still not been shown that they are more sensitive to combinations than other insects. Hence, this hypothesis, while reasonable, is not yet supported. A study like the one by Hardstone and Scott (2010), using combinations of pesticides on many different types of insects, is needed.

Phase I Studies

Studies of phase I detoxification in bees can be broken up into two categories: first, those on the metabolism of natural xenobiotics, and second, those on the mechanisms underlying pesticide metabolism. With respect to the detoxification of natural xenobiotics, it has been shown that components of honey and pollen induce p450 expression (R. M. Johnson et al., 2012, Mao et al., 2013).

In particular, the p450s (CYP6AS1, CYP6AS3, CYP6AS4, and CYP6AS10) are upregulated in the bee midgut by hive products. P-coumaric acid, a constituent of the pollen cell wall, in particular, activates detoxification pathways. This compound is also present in honey, as honey contains a significant number of pollen grains. P-coumaric acid also upregulates a CCE, esterase E4-like (Mao et al., 2013). Propolis also leads to upregulation of p450s (Mao et al., 2013). Based on these studies, it has been suggested that keeping colonies in a manner that does not involve these natural triggers for p450 induction may make bees more prone to pesticide damage. Practices that shield bees from these triggers involve artificial diets and human-made hive boxes that have less propolis on their interior surfaces than do the interiors of trees (Simone-Finstrom and Spivak, 2010, Simone-Finstrom et al., 2017, Dalenberg et al., 2020).

The body of work exploring phase I responses to pesticide exposure is much larger than that focused on natural xenobiotics. In general, many studies have now shown that both p450s and CCEs are involved in the breakdown of all major classes of pesticides (Berenbaum and Johnson, 2015). Iwasa et al. (2004), for example, used p450 inhibitors to show that these enzymes contribute to the breakdown of neonicotinoids. The use of inhibitors was central in this and other studies on this topic, and is simple in principle. If the inhibitor causes an increase in the sensitivity to the pesticide, then the enzyme(s) being inhibited is likely involved in its metabolism. Johnson et al. (2006) used a similar approach to show that bees use p450s to break down pyrethroid pesticides. They further showed that bees are particularly good at breaking down tau-fluvalinate, a major miticide. Mao et al. (2011) built on this work to show that three p450s, CYP9Q1–3, are upregulated after exposure to tau-fluvalinate and coumaphos and that both miticides are likely metabolized in the same biochemical manner. With respect to CCEs, Carvalho et al. (2013) used CCE inhibitors to show that these enzymes are involved in the metabolism of fluvalinate and cyfluthrin. Mao et al. (2011) showed that CYP9Q1–3 produce metabolites suitable for CCE cleavage. Boncristiani et al. (2012) showed that thymol, an essential oil used for *Varroa* control, causes upregulation of the p450 gene CYP6AS14.

Phase II and III Studies

Not much work has been done on phase II and III detoxification in honey bees. Nielsen et al. (2000) showed that flumethrin induces GST activity in larvae and nurses but not in foragers. Ma et al. (2011) showed that jujube pollen and nectar cause upregulation of both GSTs and p450s. Finally, coumaphos was shown to cause upregulation of GSTD, a poorly characterized GST (Schmehl et al., 2014). With respect to phase III, a handful of studies making use of inhibitors of multidrug ATP cassette proteins have shown that they are involved in detoxification (Hawthorne and Dively 2011; Guseman et al 2016).

Nicotine Breakdown

The study of how bees metabolize nicotine has become something of a model system for the study of how bees break down plant secondary compounds. Nicotine binds agonistically to acetylcholine receptors and is quite toxic to many insects. Bees encounter nicotine in the nectar of some plants, including tobacco, and it has long been known that they have considerable natural resistance to this compound. Bees fed nicotine in sugar water, for example, show little to no decrease in survivorship either as larvae or adults (Singaravelan et al., 2006, Human et al., 2014). Kohler et al. (2012) later found that nicotine is a mild deterrent for foragers, but it does not stop them from collecting nectar with this compound. They also verified that nicotine was not harmful to bees, except at exceedingly high concentrations (Hendriksma et al., 2020). Some data even suggested that low concentrations may be beneficial for bees, via some unknown mechanism. A consequence of this may be the preference for neonicotinoid-laced food shown by honey bees (Kessler et al., 2015).

Based on these studies showing limited effects of nicotine on bees, du Rand et al. (2015) conducted a metabolomic and proteomic study of nicotine detoxification in bees. They found metabolites of nicotine consistent with phase I detoxification, although no p450s were upregulated in bees fed nicotine. This suggested that the relevant p450s are constitutively expressed. With respect to phase II, they found that two GSTs were upregulated in response to nicotine treatment. They further found proteomic evidence of a stress response in bees exposed to nicotine. This included high expression of heat shock proteins, antioxidants, and increased metabolic activity for both carbohydrates and lipids. A stress response to pesticide exposure may explain some synergistic effects between pesticides and other stressors, such as pathogens, as detoxification may be energetically costly, making bees more prone to other stressors even when they do not involve the same pathways. In summary, even though nicotine is well tolerated by bees, its detoxification is metabolically costly (du Rand et al., 2017a, 2017b).

Neonicotinoid Breakdown

Given how high profile neonicotinoids are, it is appropriate to cover them separately from other pesticides. Similar to nicotine, but in a biochemically distinct manner, neonicotinoids also bind to acetylcholinergic neurons at muscular junctions, causing elevated firing and death (Gong and Diao, 2017). A series of studies have shown that neonicotinoids fall into different classes that vary in their toxicity to bees. Neonicotinoids with cyano substitutions, such as thiacloprid and acetamiprid, are less toxic to bees than are those with a nitroimine group, such as imidacloprid and thiamethoxam (figures 18.1 and 18.2). Iwasa et al. (2004) showed this differential toxicity and then used p450 inhibitors to show that these enzymes are responsible for the decreased toxicity of cyano-substituted neonicotinoids (acetamiprid and thiacloprid). Alptekin et al. (2016) later showed that several p450s, including CYP6BE1, CYP305D1, CYP6AS5, CYP315A1, and CYP30A1, were induced by thiacloprid exposure and that preexposure resulted in a temporary reduction in mortality when the bees were again challenged with this pesticide. Finally, Manjon et al. (2018) showed, using biochemical assays with all relevant p450s, that CYP9Q3 strongly metabolizes thiacloprid but has little effect on imidacloprid. They further showed that this trait is shared with bumble bees. Thus, the differential toxicity of neonicotinoids with cyano versus nitroimine substations is caused by the presence of p450s with specialized activity.

Caffeine

Like nicotine, caffeine is a plant secondary compound that is toxic to most organisms at high concentration (Mustard, 2020). Like nicotine, it is present at low doses in nectar. Wright et al. (2013) tested the intriguing idea that plants add caffeine to nectar to modulate the learning and memory of bees in a manner adaptive for the plant (and perhaps the bees). First, they found that nicotine occurs in citrus and coffee nectar. They then showed, using PER, that ingestion of field-relevant dosages, which are moderate but not high concentrations, increases long-term memory formation in bees. Bees were more likely to return to caffeine-laden nectar sources a day later. Thus, plants may manipulate the bees' neural system to encourage them to return to their flowers. Given the large number of compounds of unknown function in nectar, this a fruitful area for future work (Mustard, 2020).

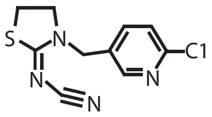
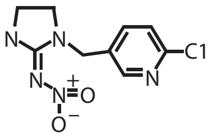
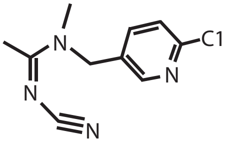
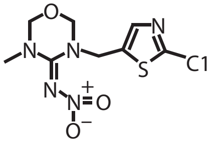
Cyano substitution	**Nitroimine group**
 Thiacloprid	 Imidacloprid
 Acetamiprid	 Thiamethoxam

FIGURE 18.1. Chemical structure of several commonly used neonicotinoids. Compounds fall into classes having either cyano or nitroimine groups (*from Iwasa et al., 2004*).

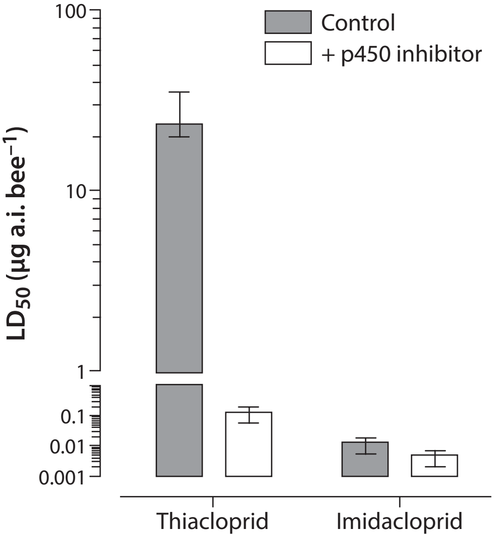


FIGURE 18.2 Honey bee LD_{50} for bees treated with thiacloprid or imidacloprid, both in the presence of a p450 inhibitor and without. Thiacloprid tolerance drastically falls in the presence of the inhibitor while imidacloprid sensitivity is unaffected (*from Manjon et al., 2018*).

Social Detoxification

It has long been known that bee sociality plays a role in detoxification (Evans and Spivak, 2010). The nature of social detoxification mainly relates to the processing and storage of food. Vannette et al. (2015) showed that the hypopharyngeal and mandibular glands, which add antimicrobial peptides and nectar processing enzymes to nectar, for example, also show upregulation of many p450s and other detoxification genes. Whether this contributes to detoxification of honey is unclear. Honey itself is acidic, and its composition is known to change over time (Liu et al., 2005). Whether this contributes to pesticide breakdown is also largely not known but should be tested (Berenbaum and Johnson, 2015). Pollen is likewise processed into bee bread, which may contain beneficial microbes and has its own unique properties that could, in principle, lead to pesticide breakdown (Gilliam, 1979, 1997). Yoder et al. (2013) showed that exposure to fungicides affects the fungal community in bee bread, potentially leading to negative health consequences. The study of the intersection between sociality and detoxification is thus ripe for future work.

Pesticides in the Hive and Comb Effects

Not long after the use of miticides in the hive became pronounced in the 1990s, it was shown that these compounds are lipophilic and build up in the wax comb (Lodesani et al., 1992, Bogdanov et al., 1998, Bogdanov, 2006)). Early work also showed that pesticides, including those not targeted to honey bees, such as fungicides, could also build up in bee colonies, particularly in bee bread (Kubik et al., 1999, 2000). The colony collapse disorder (CCD) problem brought both of these issues to the attention of many, with a surge of new studies attempting to better understand the problem in the current climate of widespread neonicotinoid use.

In France, where pressure to ban neonicotinoids was intense earliest, a series of studies attempted to document how contaminated hives were with various classes of pesticides. This work largely supported earlier studies by showing that miticides were the most common pesticides in the hive and were highest in wax (Chauzat and Faucon, 2007). Neonicotinoids were also found in the pollen of most samples, and fipronil, a commonly used phenylpyrazole, was found at lower but still significant concentrations (Chauzat et al., 2006). A study by Chauzat et al. (2009) looking for correlations between levels of pesticides in hives and colony survivorship found little to no evidence for a link. In contrast to this work, a large-scale search for pesticides in hives in the United States showed more serious levels of contamination (Mullin et al., 2010). Miticides were again the pesticides at highest levels and were mainly in wax, while fungicides were prevalent in pollen, but levels overall were higher and the rate of multiple contamination was significantly worse than in France. Six different pesticides were found on average per sample, for example, and while levels were generally in the range expected to cause sublethal effects from a single-pesticide perspective, when all the pesticides (and fungicides and herbicides) in a sample were added together, the total levels were quite high. Later studies have largely confirmed these findings (Pettis et al., 2013), although significant variation has been found. Traynor et al. (2021) recently conducted a nationwide survey of in-hive pesticide levels in pollen across the US. The average sample (with a sample size over a thousand) contained a mean of 2.78 compounds, and evidence for exposure to about 120 different pesticides was found. Most samples contained low-risk levels of xenobiotics, but high-risk samples containing many pesticides (both insecticides and miticides) were not uncommon.

Follow-up experimental studies to Mullin et al. (2010) have shown that the chemicals found in combination in wax can be more damaging to bee health than previously thought (R. M. Johnson et al., 2013a, Yoder et al., 2013, Zhu et al., 2014, Doublet et al., 2015, Ravoet et al., 2015a, Mao et al., 2017). Wu et al. (2011, 2012), for example, found that bees reared in heavily contaminated comb have significantly higher mortality as larvae and reduced longevity as adults. Zhu et al. (2014) tested the four most common pesticides in hives singly and in combination for their effects on larval development. All four had negative effects in isolation, and synergistic effects were found between fluvalinate and chlorothalonil and between coumaphos and chlorothalonil. A common solvent used in the pesticide industry, N-methyl-2-pyrrolidone, also had a negative effect on larval health. Finally, studies showing entombed pollen—highly contaminated pollen sealed up by the bees and not used—suggests that, in some cases at least, bees can recognize contaminated bee bread and avoid it (vanEngelsdorp et al., 2009a). Dolezal et al. (2016a) likewise found that bees prefer to feed on pollen not contaminated with pesticides.

Insecticide Sublethal Effects

There is now a large body of literature showing sublethal effects on many aspects of honey bee health (R. M. Johnson et al., 2010, Blacquiere et al., 2012, Berenbaum and Johnson, 2015, Glavan and Bozic, 2013, Gong and Diao, 2017, Dai et al., 2019). There is some consensus that many classes of pesticides, including neonicotinoids, cause significant impairment of bee health when encountered at levels far below their LD_{50}. We discuss a handful of the clearest studies across several topics, including longevity, learning and memory, and foraging. Most of this work is laboratory based, and a critical need is to show that the behavioral deficits have significant effects in the field.

As for many of the topics in this chapter, it is the case that most of the basic results were known prior to the arrival of CCD, and new studies have mainly clarified and expanded on older work. The most straightforward type of sublethal effect is a decrease in longevity at either the individual bee or colony level. Early work showed that bees treated with sublethal doses of pesticides have decreased survivorship; however, the effects are pesticide specific (even within pesticide classes) and differ between larvae and adults (Atkins and Kellum, 1986, Mackenzie and Winston, 1989). More recent work has addressed whether sublethal doses at field-realistic levels (which are sometimes quite low) also affect survivorship. The results are mixed. Dively et al. (2015), for example, fed imidacloprid-treated pollen to colonies and found little to no effect at the most common field-relevant dosages. They did, however, find negative effects at the highest dosages seen in the field. They also found negative effects on queens, which could be critical given the role of queen failure on colony survivorship. Wu-Smart and Spivak (2016) also fed imidacloprid (via syrup) to colonies and found effects on queen laying rate and colony development. Finally, a recent study by Traynor and Lamas (2021) suggests a link between long-term exposure to insecticides and brood loss and between long-term exposure to fungicides and queen turnover (a major cause of colony mortality). Similar results were found by Fisher et al. (2021).

A number of studies have explored sublethal behavioral effects thought to affect fitness (Desneux et al., 2007, Blacquiere et al., 2012). Most of these studies have explored memory deficits and used the classic PER assay (see chapter 7) (Weick and Thorn, 2002, Decourtye et al., 2004, Aliouane et al., 2009, Bell et al., 2020, Ludicke and Nieh, 2020). Most studies focused on neonicotinoids, but recent work has shown similar effects for other newer classes of pesticides, including butenolide, an acetylcholine receptor agonist (Tosi and Nieh, 2019). Declines in motor function, phototaxis, communication, and general activity have also been found, as has decreased ability to avoid predators (Eiri and Nieh, 2012, Tosi and Nieh, 2017, Tosi et al., 2017a, Tan et al., 2014, Zhang et al., 2020). Studies have also shown altered sucrose responsiveness in bees treated with pesticides (El Hassani et al., 2008, Aliouane et al., 2009). This is interesting, as sucrose responsiveness is known to affect foraging decisions (Page et al., 2012, Rueppell et al., 2006).

The body of work on foraging and pesticides is large but also somewhat contentious. The main issue relates to studies using higher than field-relevant dosages. High-profile examples in which the study is biased toward finding an effect could be given (Henry et al., 2012), but as the problem is not systemic to the field, it is not necessary to dwell on this issue. There are properly conducted studies, and some show clear effects at field-relevant dosages. The overall results are a mixed bag, however. Early work showed that foragers treated with sublethal doses of permethrin, a pyrethroid, foraged at a reduced rate (Cox and Wilson, 1984, Vandame et al., 1995). Later work by C. W. Schneider et al. (2012) used radio frequency ID (RFID) tags to track foragers as they foraged from feeders laced with imidacloprid or clothianidin. No significant effects were found at field-relevant dosages, although at higher than natural dosages foragers slowed down. Yang et al. (2008), in contrast, found that bees fed imidacloprid at a feeder foraged at a slower rate. It may be that the differences in dosage explain the variable results, or they could be explained by differences in the bees used in the experiments. It may be that other colony stressors varied between the bees used in the various studies, and this makes comparison problematic (Tosi et al., 2017a, 2017b). These issues are relevant across the board for the many instances of lack of corroboration found in pesticide studies on bee health.

Miticide Sublethal Effects

It is best to cover miticides separately from insecticides targeted to agricultural pests. This is for two reasons. First, honey bees are intentionally exposed to miticides, so there is no question as to whether they suffer their effects in the real world. Second, miticides are chosen because they are supposed to be safe for bees, or at least safe enough to justify their use (Lindberg et al., 2000, R. M. Johnson et al., 2013b, Rangel and Tarpy, 2015). In other words, they should be quite damaging to the mites and at least tolerable to the bees. The procedures used to formally test for this are often lacking, however, leading to research post–widespread use on the health effects on bees (Mattila et al., 2000, Pettis et al., 2004, Collins et al., 2004, Boncristiani et al., 2012, Garrido et al., 2013). The most serious effects of miticides are in the context of interactions with each other and other xenobiotics, but we cover that topic in the next section. Here we focus on sublethal effects that are the direct consequence of each miticide alone.

A large variety of compounds in different classes have been used through the years to control Varroa mites. The most common miticides in wax are still tau-fluvalinate and coumaphos, in spite of the fact that these two compounds are no longer in widespread use due to the evolution of resistance by Varroa mites (Elzen et al., 1998, 1999, Pettis, 2004). Until commercial suppliers, and beekeepers, stop using old wax, it will thus be necessary to consider these compounds. Both compounds decrease queen size and fitness and interfere with normal drone reproductive behavior (Rinderer et al., 1999, Haarmann et al., 2002, Collins et al., 2004, Burley et al., 2008). Amitraz and fenpyroximate were commonly used miticides for a while. They are quite different compounds, but they share the fact of having been the subject of little research. Amitraz, at high concentrations, may decrease survivorship and decrease resistance to viral infection, while fenpyroximate may increase reactive oxygen species (damaging agents within cells), but little else is known (Hillier et al., 2013, O'Neal et al., 2017). The essential oils thymol and menthol are commonly used miticides that are known to affect worker and queen survivorship (Ellis and Baxendale, 1997, Whittington et al., 2000, Floris et al., 2004). Finally, two organic acids are also commonly used miticides: formic and oxalic acid. Formic acid may affect adult and brood survival (Fries, 1991, Underwood and Currie, 2003, Gregorc et al., 2004), while oxalic acid may alter queen quality and brood survivorship and affect learning and behavior (Higes et al., 1999, Hatjina and Haristos, 2005, S. Schneider et al., 2012). Most miticides elevate gene expression associated with detoxification pathways (Gregorc et al., 2012).

Adjuvant Effects on Bees

Regulatory approval for pesticides is based on studies of the so-called active ingredients, typically when tested in isolation (Mullin et al., 2015, 2016). In addition to the need for testing for interactions between active ingredients, it is now clear that the supposedly inactive ingredients can also be toxic (Chen et al., 2018). Adjuvants, in particular—compounds with many purposes, including increasing the penetration and spread of compounds over plant surfaces—have been the subject of several studies. An early study showed, using PER, that commonly used organosilicone-based surfactant adjuvants disrupt learning and memory in a manner similar to what was recorded for pesticides (Ciarlo et al., 2012). Chen and Mullin (2013) then showed that organosilicones are present in honey bee wax and in most pollen samples. This is troubling, and not surprising, given that these compounds are commonly used in almond orchards. Fine et al. (2017) followed up this work with a study in which an organosilicone adjuvant product was fed to developing larvae, either in the presence of a viral cocktail of four common bee viruses or in its absence. The adjuvants alone did not affect larval survivorship much, but when administered in conjunction with viruses, they greatly increased viral pathogenicity. Black queen cell virus, in particular, was elevated synergistically in the presence of the adjuvant. Finally, Chen et al. (2018) recently showed that feeding common organosilicones to adult bees also decreases their survivorship. In sum, although work is just beginning on this topic, it is possible (even likely) that many serious interactions (and direct effects) associated with adjuvants and other colony stressors may be found.

Pesticide Interactions

We cover interactions between pesticides and other colony stressors, like food stress, parasites, and pathogens, in the final chapter. Here we review work on interactions between various classes of synthetic xenobiotics: pesticides, fungicides, herbicides, solvents, and adjuvants (R. M. Johnson et al., 2010, 2013a).

Before we review the work to date on honey bees, it may also be useful to describe the basic mechanisms underlying interactions between pesticides (Glavan and Bozic, 2013). As for the many other topics in this chapter, most of these were worked out prior to the CCD crisis, and mainly in other insects. Recent work has applied these principles to honey bees and the most relevant xenobiotics they encounter. The mechanisms fall into three main categories. The first cause of an interaction between two pesticides depends on the same detoxification enzymes underlying the metabolism of both. If the same p450 enzymes are needed to break down both compounds, for example, then there may be competitive interference when both are present. The second category relates to inhibition of the relevant detoxification enzymes. To use p450s again as an example, if a xenobiotic inhibits a p450 (or many of them), it will cause massively increased toxicity of all those compounds normally metabolized by that p450. Finally, the third mechanism is associated with the different xenobiotics interfering with the function of the same neurons. One compound may interfere with neurotransmitter function, for example, while a second may disrupt neuromodulators of the same neurons, greatly affecting their excitability. The first two mechanisms are easy to show, and we have clear examples, while the last is poorly understood but is the most likely explanation for the more complex and difficult-to-understand patterns of interaction.

Many early studies showed interactions between insecticides and fungicides. These effects were shown to be caused by ergosterol-biosynthesis-inhibiting (EBI) fungicides interacting with pyrethroid or other classes of pesticides (Pilling and Jepson, 1993, Vandame and Belzunces, 1998, Schmuck et al., 2003). The mechanism is simple in that EBI fungicides target p450s in fungi, and they also have the ability to decrease function of insect p450s. With respect to interactions between pesticides, Ellis and Baxendale (1997) showed that tau-fluvalinate, the most common pyrethroid acaricide, interacts with another pyrethroid, bifenthrin, probably due to a shared p450 used to detoxify both. Johnson, in a series of studies, expanded on this work. Coumaphos and tau-fluvalinate were first shown to interact agonistically, likely due to being detoxified by the same p450, suggested by studies using p450 inhibitors (R. M. Johnson et al., 2009). A follow-up study comprehensively looked for interactions across many relevant xenobiotic classes (R. M. Johnson et al., 2013b). Many interactions were found, some of which are explained by the common use of p450s, while others, such as a one-way interaction for amitraz and other compounds, are not yet understood. The take-home message is that, based on this work and that of Mullin et al. (2010), the opportunity for and reality of interactions between disparate compounds is striking.

Sublethal Effects Based on Real-World Field Studies

Other than the work on miticides, most of the work reviewed thus far has been based on lab studies. Work conducted in the field with commercial hives is clearly needed to validate such results. Early work showed low levels of neonicotinoids in pollen and nectar collected from the field, and it was assumed that these levels were too small to affect bee health. Several field trials have supported this expectation. Pilling et al. (2013), for example, conducted a four-year field study of bees exposed to thiamethoxam-treated crops in France and found no adverse effects on bees (figure 18.3). Genersch et al. (2010) in a large-scale study in Germany found similar results; no adverse effects on bees located near crops treated with neonicotinoids. Many more studies have found weak to no effects of neonicotinoids on bees in the field (Cutler and Scott-Dupree, 2007, Nguyen et al., 2009, Pohorecka et al., 2012). In contrast, some recent work has found that neonicotinoids do affect honey bee health. Woodcock et al. (2017) found that proximity to neonicotinoid-treated fields in the UK and Hungary, but not Germany, adversely affected colony survivorship. Likewise, Sandrock et al. (2014) found that queens in the field are very sensitive to neonicotinoids, which is important given that queen loss is a major driver of colony

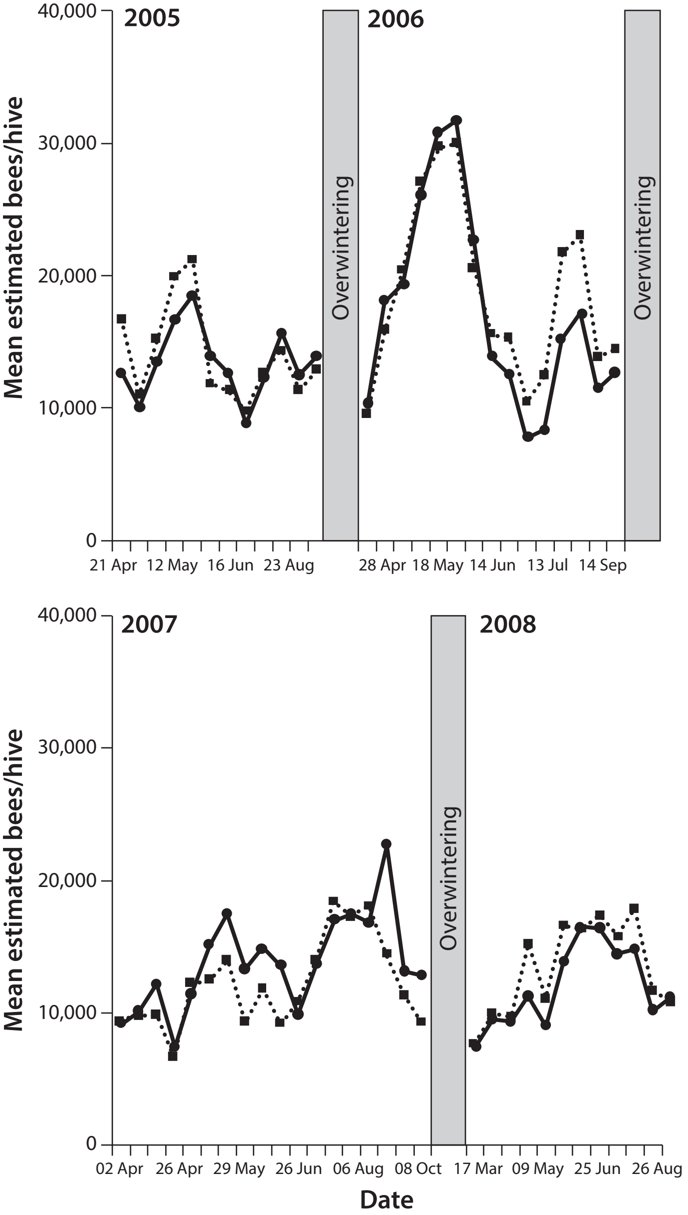


FIGURE 18.3. Colony strength over four years between hives exposed to thiamethoxam-treated fields (dotted lines) or control colonies (solid lines). No significant overall differences were found (*from Pilling et al., 2013*).

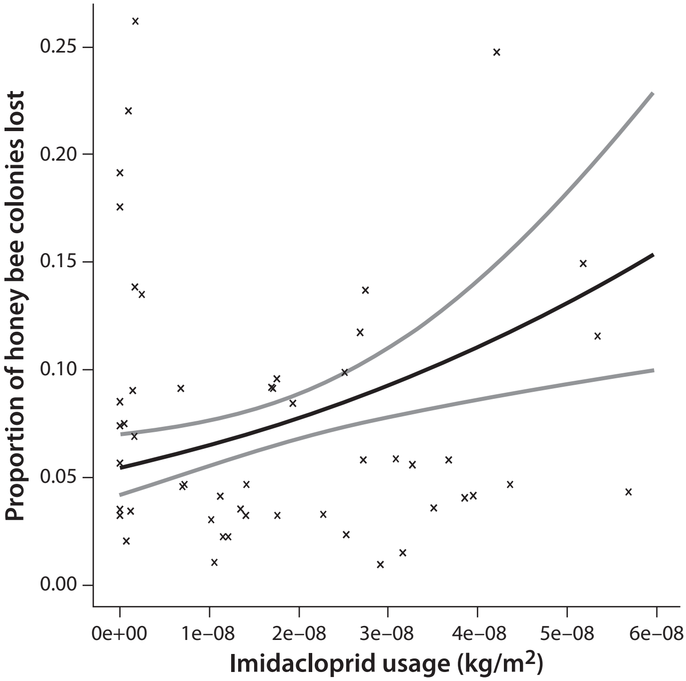


FIGURE 18.4. A significant association between imidacloprid usage in the agricultural landscape and honey bee survivorship over much of the UK from 2000 to 2010 (*from Budge et al., 2015*).

losses. Another long-term study showed a nice dose-dependent relationship between neonicotinoids in pollen and colony survivorship, but found that neonicotinoids could only cause significant mortality at the highest levels found in the field and not at typical levels (Dively et al., 2015). Budge et al. (2015) showed a correlation between neonicotinoid usage and colony losses over parts of the UK (figure 18.4). Finally, Tsvetkov et al. (2017) found that proximity to corn, a source of neonicotinoid exposure, increased worker and queen mortality, particularly when also exposed to fungicides.

As this brief review makes clear, the case is still open as to whether neonicotinoids cause significant honey bee losses in the field. It may be that they invariably do, it may be that they only do so in the presence of other stressors, or it may be that they do not cause significant losses. Given the large number of studies currently ongoing, an answer will probably begin to emerge within the next several years.

19

Honey Bees as Managed Pollinators

There is currently great interest in the biology of pollinators growing from the colony collapse disorder (CCD) crisis, which elucidated for many how dependent agriculture is on bees. Research has been along two fronts. First, more funding has flowed into the labs of those who study the various problems facing honey bees, and second, more interest, and funding, has gone into developing other bee species as alternative managed pollinators. In this chapter, we introduce the reader to this field from a honey bee–centric position. We explain why some forms of agriculture are so dependent on managed pollinators and why the honey bee is the cornerstone of managed pollination. We explore why our dependence on the honey bee is unlikely to change unless we change how we grow food. We also discuss the contemporary state of commercial beekeeping. We conclude with a brief review of large-scale losses of managed colonies.

Relevant Pollination Biology

Pollination refers to the movement of pollen from the male part of the flower (called the anther) to the female part (called the stigma). It is simple in principle, but the bewildering diversity of plant sexual anatomy can make the topic confusing (Johnson and Steiner, 2000, Ricketts et al., 2008, Garibaldi et al., 2011). We introduce the basics of this subject, focusing on those aspects that are important for understanding the role played by honey bees as wild and managed pollinators.

Generally speaking, those plants that require animal vectors for reproduction are in a mutualistic relationship with them. The plant provides food in the form of nectar and pollen, and the animal transports pollen. A key point is that it is only some of the angiosperms, or flowering plants, that require animals to transport their pollen (Fenster et al., 2004). Conifers, for example, are wind pollinated, and so are many grasses, which are angiosperms. Most of our major crops, such as corn, wheat, and rice, are grasses that are wind pollinated.

Many animals can serve as pollinators, but bees are the clade most closely tied to plants in this way (Michener, 1974, 2000). Nearly all bee species feed exclusively on pollen and nectar, and all have adaptations specializing them to act as pollinators. The hairy bodies of bees, for example, ensure that pollen sticks to them and is transported from flower to flower. Wasps, another group that visits flowers for nectar, have smooth bodies and are hence less efficient transporters of pollen. The same is true of ants, beetles, and many other insects who visit flowers. Generally speaking, the bees, some flies, and butterflies are the major insect pollinators.

There are a few aspects of basic plant biology that are important to understand with respect to how bees are used as managed pollinators (Fryxell, 1957, Knight et al., 2005). First, some flowers require special handling for pollination. The most important two cases refer to plants requiring buzz pollination and alfalfa, which has a unique pollination mechanism. For plants like tomatoes that require buzz pollination, the flower is shaped like a salt shaker and the pollen must be vigorously shaken out. The bee does this by grasping the flower and buzzing her flight muscles. Most bee species will do this, but honey bees, and a minority of other species, will not. Hence, the commercial pollination of tomatoes, and related plants, is mainly done by bumble bees. Alfalfa has a springing mechanism in its flowers such that when a bee visits it, the spring is released, and the stamen hits the bee in the head (Brink and Cooper, 1936, Bosch and Kemp, 2005). This action is good for the plant, as it ensures that the bee is doused with pollen, but bad for the bee, which has to groom most of the pollen off before she can fly away, thus wasting her time. After a few jolts, honey bees stop visiting alfalfa flowers. Hence, this important crop is typically not pollinated by honey bees. Alfalfa leaf cutter bees, a managed solitary bee species, largely pollinates this crop. The alkali bee, *Nomia melanderi,* another managed solitary bee, also does the job in places where this bee is native, mainly the Pacific Northwest (Bosch and Kemp, 2005, Cane, 2008).

Two additional plant characteristics important for managed pollination relate to nectar concentration and self-incompatibility. With respect to nectar concentration, many important crops produce nectar of low sugar content (Free, 1968a, 1980). For these crops, the surrounding dandelions, and other wildflowers, are more attractive to bees. This causes farmers to attempt to eliminate all noncrop plant life (weeds) in the fields to ensure that bees visit mainly the crop. Areas practicing this form of farming are food deserts whenever the crop is not in bloom, which is the majority of the time. With respect to self-incompatibility, most plants are not self-fertile. Even plants that have male and female flowers on the same plant are usually not able to pollinate themselves due to molecular self-incompatibility mechanisms or variable timing of male and female flower maturation (Takayama and Isogai, 2005). Plants that are self-infertile require more managed pollinators because the pollen must move farther. Plants that are self-fertile, or seedless, either do not require pollinators or require very few (Pandolfini, 2009). Hence, much of the burden with respect to demand for managed pollinators could be alleviated if more self-fertile crops were grown. A difficulty here is that sometimes a self-fertile variety is less desirable in the marketplace than a self-infertile variety. This is the case for almonds, the most important crop from an economic perspective (Godini et al., 1992, Kester et al., 1994). Finally, it is often the case that crops that do not require pollinators, because they are self-fertile and can pollinate themselves, still benefit from cross-pollination via pollinators, as it increases genetic diversity in the fruit, producing a larger and better crop (Klein et al., 2007).

What Crops Are Dependent on Bees?

Crops dependent on honey bees, and other managed pollinators, are responsible for billions of dollars in economic activity each year (Winfree et al., 2011, Kevan and Phillips, 2001). About 400 crops worldwide are dependent on bees for pollination (Calderone, 2012). It is thought that about a third of our food comes from pollinated crops (Klein et al., 2007, Allsopp et al., 2008, Gallai et al., 2009). This includes both those crops directly pollinated and those that indirectly benefit from pollination. Many vegetables, for example, do not require pollination because we eat the plant, not the fruit. However, growing the vegetables requires seed production, which does require pollinators. Likewise, alfalfa and other important animal feeds depend on pollinators for seed production. With respect to food we eat that is directly pollinated, it is mainly fruits and nuts.

Why Do We Need Managed Pollinators?

Nature seems to abound in wild pollinators, so a natural question is, Why do we need so many managed pollinators (Klein et al., 2007, Williams and Winfree, 2013)? There are three main reasons why natural populations of pollinators are typically insufficient to meet the demands of modern farms. All three factors relate to farming practices centered on maximizing productivity. It is therefore modern industrial farming that requires managed pollinators, and the degree of industrialization and the dependence on managed pollinators are tightly connected.

All three of the issues we discuss involve practices that make an industrial farm a hostile landscape for wild bees and other pollinators (Kremen et al., 2002, Biesmeijer et al., 2006, Potts et al., 2010a, Vanbergen et al., 2013, Kennedy et al., 2013). First and foremost, modern farming is often based on monocultures—large fields of one crop. In a wild landscape, in contrast, many hundreds or thousands of plant species are growing, and they flower at different times of the year. There is thus a steady and moderate number of flowers present year-round (Kremen et al., 2002). This can support a diverse population of pollinators through the year. A monoculture, in contrast, only produces food for a couple of weeks. Further, even when flowers are in bloom, there are so many of them that a wild population of pollinators would be unlikely to be able to pollinate all of them.

The second issue has to do with heavy tilling of the soil. This destroys nesting sites for many bee species, many of which are ground nesters (Michener, 2000). Further, monoculture prevents the diversity of shrubs and bushes that allows for nesting in twigs or under branches. In short, most bee species either live in the ground or in twigs, and large monocultures and industrial farming practices destroy these nesting sites.

The third issue relates to the heavy use of pesticides (Mullin et al., 2010, Johnson, 2015, G. R. Williams et al., 2015, Wu-Smart and Spivak, 2016, Sanchez-Bayo et al., 2016). Modern farms make extensive use of pesticides to kill the many pest insects that would otherwise consume a considerable portion of each crop. The enormous potential for pests to consume much of the crop is another consequence of monoculture, as diverse farms are less prone to population explosions of destructive pests (Beddington et al., 1978, Lawton, 1983). Pesticides not only kill the pests, however; they also kill the beneficial insects, like pollinators and predators of the pests (Amano and Haseeb, 2001, Stokstad, 2013).

Why the Honey Bee?

The previous section made it clear that industrial farms, particularly those growing crops in monoculture, are hostile places to the insects that live in them. Given this, the reason for modern agriculture's dependence on managed pollinators should be clear, along with why the honey bee is so ideally suited. Essentially, the honey bee can be moved to where they are needed in any numbers necessary and then moved away when no longer needed. Hence, an orchard that needs millions of pollinators on Tuesday can call a commercial beekeeper a few days before and have them delivered. Then when pollination is complete, and the farmer wants to begin spraying pesticides, the honey bees can be driven away. A honey bee colony can thus be a cog in the industrial farming machinery. It is unlikely that industrial-style farming could even have evolved in the absence of *Apis mellifera*.

The honey bee has other characteristics that make it a valuable managed pollinator. Their large colony size and the fact that they live in boxes and can be managed (fed, medicated, and so forth) adapt them for use in the assembly line process of modern agriculture. It should be apparent why it is so difficult to use most other bee species in the same way. Many bees nest in the ground as solitary insects. They obviously cannot be moved around. Even bees that nest in twigs, which can be mimicked with artificial tubes and are possible to establish near a farm, cannot readily be moved once they are nesting. Bumble bees, which can be moved around, have small colony sizes and an annual life history, which makes them less suitable for large-scale agriculture. In short, some solitary bees, such as the alfalfa leaf cutter bee, can be managed to pollinate large crops, but it is typically only for crops for which honey bees cannot be used.

With respect to crops that honey bees will work, it is unlikely that a solitary bee will be found that is even remotely as amenable as a managed pollinator. This is assuming, of course, that monoculture and all the other hallmarks of modern agriculture are not changed. In short, to farm in an industrial fashion requires a bee like the honey bee. To move away from the honey bee (or a bee species like it, such as the Asian honey bee or some stingless bee species) requires changing the way we grow crops. Of course, a great many people think we should do precisely this, as industrial agriculture has many negative consequences for the environment and the economy (Thrupp, 2000, Robertson et al., 2004, Reganold et al., 2011).

Commercial Beekeeping

Pollination and Honey Production

Commercial beekeeping is an economic activity, so why cover it in a book about honey bee biology? Basically, it is necessary to give a brief introduction to this field because so much of applied honey bee biology, covered in the last three chapters, is based on solving problems that occur in commercial beekeeping. This section takes a US-centric position, although much of the discussion is relevant elsewhere.

A commercial beekeeping operation is typically thought of as one running more than 300 colonies for profit. Many commercial beekeepers have thousands or even tens of thousands of colonies, however. Historically, commercial beekeepers made their income from honey production with pollination an important additional source of revenue. Presently, this has flipped with pollination being the major breadwinner for many operations. The reasons are twofold. First, beekeeping is a labor-intensive activity, and countries with cheaper labor costs are able to produce honey more cheaply than are US beekeepers. Much honey production has thus moved abroad to countries like China, Mexico, and Argentina (Daberkow et al., 2009, vanEngelsdorp and Meixner, 2010, Burgett et al., 2010). Second, the pollination industry took off in a major way with the growth of the almond industry in California, and beekeepers are now able to charge more for pollination services than ever before (Sumner and Boriss, 2006).

The almond crop alone now dominates the field of commercial pollination in terms of income generation, so some knowledge of this crop is necessary to understand the industry and how bees fit into it (plate 16). With respect to the logistics of pollination, almond pollination is challenging for many reasons. First, the number of orchards is huge, requiring over 1.5 million colonies each year (vanEngelsdorp and Meixner, 2010, Champetier et al., 2015). California, where the entire industry is located, only has a fraction of the colonies needed, so most must be trucked in from across the country. Second, this crop blooms early in the year, often in February. This is a time when colonies are just beginning brood rearing and still overwintering in most of the country. Getting a strong colony at this time of year thus typically involves feeding of artificial diets, something thought to potentially have health consequences. Third, the process of allocating 1.5 million hives to orchards in California's Central Valley involves crowding bees into vast holding yards, and there are concerns that this may facilitate the spread of disease. Fourth, the almond industry is rather new, and it is not clear that it is sustainable, primarily due to its massive demand for water in a state with frequent water shortages. Finally, the reader might wonder if the beekeepers make a good honey crop from almonds. The almond orchards do provide a good honey flow in years in which it is warm enough for good foraging. However, almond honey does not taste good (to most people), so the bees are allowed to keep it.

A final note on commercial beekeepers relates to the notion of "migratory beekeeping." This refers to large-scale beekeepers who move their operation from crop to crop, often across states, to either make honey or provide pollination services. A common approach, for example, is to start with almonds in the Central Valley, then move to the Pacific Northwest for cherries or apples, and then to the Dakotas to make honey in the clover fields. There are other schedules followed by beekeepers in other parts of the country.

Honey Bee Production (Queens and Packages)

Queen rearing and package production of honey bees are occupations that have been around for many years. In the past few decades, however, they have grown in scope and changed somewhat in nature (vanEngelsdorp and Meixner, 2010). This is mainly because colony losses now require the purchase of new bees at a much higher rate than ever before. The arrival of Africanized bees to the US also radically changed the industry. Packages and queens are now routinely purchased to replace those European colonies taken over or displaced by Africanized bees. Further, many large-scale breeders of bees were in locations now dominated by Africanized bees, such as Texas. This has shifted much of bee breeding to Northern California and a few other places. In sum, bee production is now a major source of income for a number of commercial beekeepers in a few locations. Some have expressed concern that having such a large percentage of the bees in use around the country coming from a few genetic sources may lead to loss of genetic variability (Cobey et al., 2012). This does not seem to have happened yet (Harpur et al., 2012, 2015), but the problem should be monitored as it could occur in the future.

Colony Collapse Disorder

Colony collapse disorder, or CCD, refers to a wave of colony losses that occurred around 2006 (Cox-Foster et al., 2007, vanEngelsdorp et al., 2009b, Ellis et al., 2010, Williams et al., 2010, Dainat et al., 2012). Some commercial operators noticed sudden large-scale losses, which they reported were not due to the typical reasons (*Varroa*, starvation, etc.). Early researchers coined the term CCD for this phenomenon, and it received massive media attention (vanEngelsdorp et al., 2007, 2008, Cox-Foster et al., 2007). It was described as consisting of (1) the rapid loss of adult bees with brood remaining, (2) lack of dead workers in the nest or immediately outside, and (3) delayed attack by pests or other honey bees. Although some studies identified particular pathogens or parasites as being associated with CCD, all such research was equivocal (Cox-Foster et al., 2007, Higes et al., 2009, Maori et al., 2009, Tokarz et al., 2011).

CCD seems to be scarce at present. What exactly caused it and how widespread it was are thus not known. What CCD ultimately did, however, was greatly increase the attention of the public and the scientific community on the issues facing honey bees and other pollinators. With or without CCD, a high percentage of losses occur each year in commercial beekeeping operations (vanEngelsdorp et al., 2011, 2012). Losses are greater than in the past when beekeeping was logistically easier (prior to *Varroa*). The effort and money spent recovering from losses decreases income for beekeepers, although, ironically, losses can in some circumstances cause a shortage of supply and higher prices for beekeeping services, making it difficult to calculate the net effect on profitability.

Are Honey Bee Populations Declining?

Discussion of CCD in the popular press gave rise to the widespread belief that honey bee populations are failing and that there is an ecological crisis threatening bees. This a complex issue and, depending on how one defines "declining," this now common belief can be true or false. Essentially, this is an issue in which simple conclusions and prescriptions are found lacking on close inspection. At the heart of the matter, however, there are serious concerns that face managed honey bees. We leave aside the issue of wild bees, which are clearly falling in population due to anthropogenic changes to the environment (Kremen et al., 2002, Biesmeijer et al., 2006, Goulson et al., 2008).

Figure 19.1 is now a common sight at talks to the public about honey bee losses (vanEngelsdorp and Meixner, 2010). This is the US population of honey bees through time since the mid-1940s. What should be immediately apparent are two things: first, the losses started after World War II, and second, CCD did not happen until 2006. Further, there are not sudden large-scale losses but

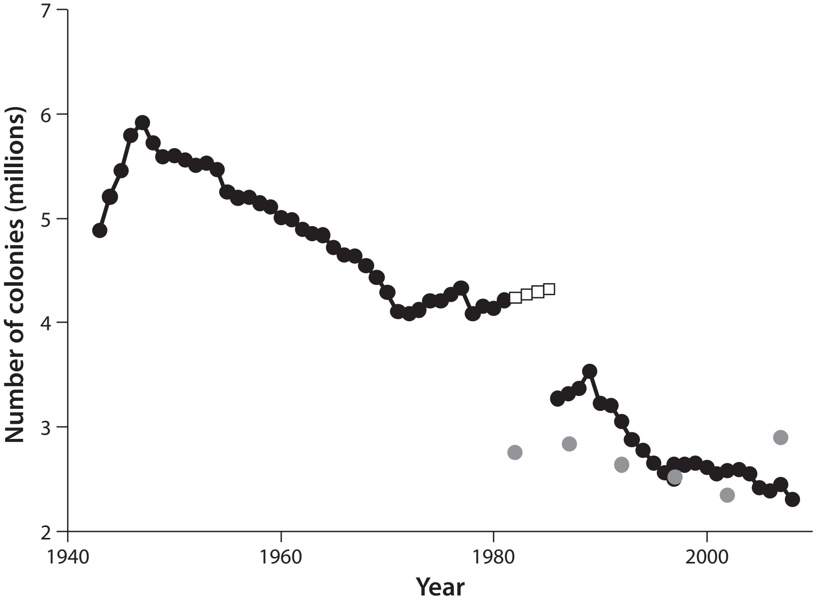


FIGURE 19.1. Number of managed colonies in the US from 1944 to 2008 (*from vanEngelsdorp and Meixner, 2010*).

rather a slow winding down of the population size. The sudden drop in the early 1980s was caused by a change in the accounting methods used by the USDA. What is going on here in general does not have much to do with pathogens, parasites, or pesticides (though these things are important) but rather with land use patterns and the economy (Smith et al., 2013). The United States used to have millions of small farms, and a high percentage of these farmers kept bees. As big agrobusiness took over American agriculture, they created gigantic farms producing a handful of staple crops in monocultures. These corporations had no use for bees. Hence, the managed bee population fell (Daberkow et al., 2009, Burgett et al., 2010).

Other factors are also important for explaining figure 19.1, such as the partial movement of the honey industry overseas. The best predictor of the population size of honey bees, in fact, is the price of honey, detailed in figure 19.2 (vanEngelsdorp and Meixner, 2010). When beekeeping is profitable, more people do it and the honey bee population increases (Sumner and Boriss, 2006). Figure 19.3 shows the global managed honey bee population, which has been rising through time. Drilling down shows a complex pattern of rise and fall country by country, mostly dependent on the economics of commercial

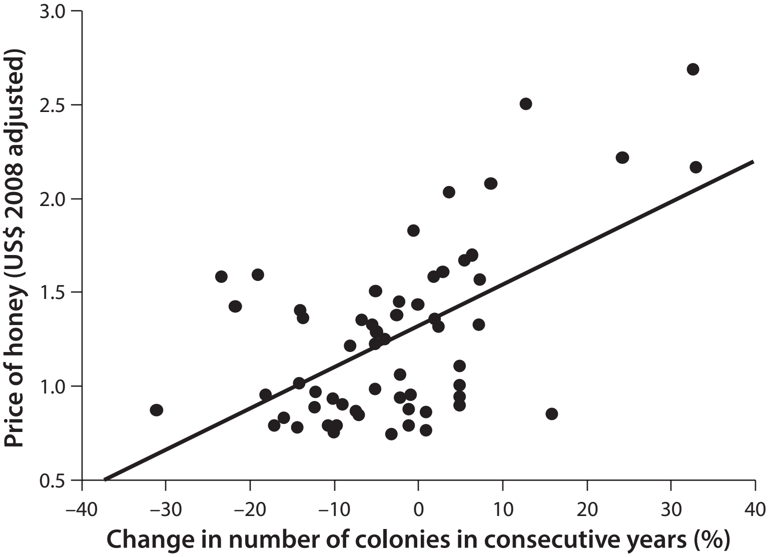


FIGURE 19.2. Strong positive relationship between the price of honey and the change in the number of colonies across years (*from vanEngelsdorp and Meixner, 2010*).

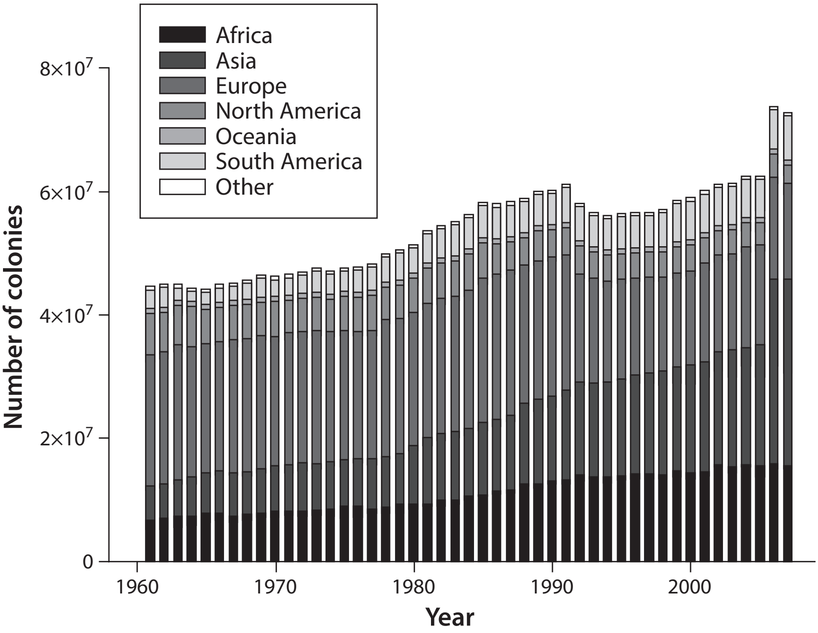


FIGURE 19.3. Global population of honey bees since 1960 (*from vanEngelsdorp and Meixner, 2010*).

beekeeping (reviewed in vanEngelsdorp and Meixner, 2010). This economic cornerstone to managed honey bee population dynamics is often missing in presentations of this issue to the public by biologists, which is unfortunate, as it is misleading to focus only on biological and ecological factors when the patterns referenced above have additional causes related to politics and economics.

Bee Losses in the United States and Europe

Although honey bee populations are probably not falling due to the commonly believed reasons, colony losses are nevertheless quite real and important. There is currently much interest in the causes of losses (particularly those over winter), as they appear to be larger than at most periods in the past (vanEngelsdorp et al., 2008, 2011, Nguyen et al., 2010, van der Zee et al., 2012, K. V. Lee et al., 2015, Kulhanek et al., 2017). We review now the main suspects.

Varroa

The Varroa mite is the clearest cause of large-scale honey bee losses (Guzman-Novoa et al., 2010, Dahle, 2010, Le Conte et al., 2010, van Dooremalen et al., 2012, Francis et al., 2013). There is no question that when Varroa mite control measures fail, as they have been doing for the last 10–15 years, mites and their associated viruses will kill colonies in great numbers. The clearest data showing this are studies in Canada and Norway, which identified the mite as the main cause of colony losses (Guzman-Novoa et al., 2010, Dahle, 2010). The work reviewed in chapter 17 showing the rapid population crashes in Hawaii after the mite was introduced are also convincing (Martin, 2010, Martin et al., 2012). Siede et al. (2018) conducted a study in which multiple stressor levels were recorded over a year. *Varroa* infestation turned out to be the best predictor for colony strength, with pesticide exposure having no significant effect at field-relevant doses. Perhaps the most convincing of all with respect to the key role played by the mite is the fact that large-scale bee losses have not occurred anywhere the mite is not present. Australia, and those Hawaiian islands that do not yet have mites, have shown no large-scale bee losses, in spite of the fact that they use the same pesticides as elsewhere and all the other stressors affecting bees are present (Roberts et al., 2017).

Pesticides

We reviewed the many ways that pesticides adversely affect bee health in the previous chapter. In short, it is obvious that pesticides are poisons that are bad for bees. However, it is not so easy to clearly show that pesticide use is leading to large-scale losses of managed bees in the field. The studies to date are ambiguous—some show significant effects, but others show no effect at all (Nguyen et al., 2009, Genersch et al., 2010, Pilling et al., 2013, Sandrock et al., 2014, Woodcock et al., 2017). Further, why are bees not dying in large numbers in Australia, given their large-scale use of pesticides in all classes, including neonicotinoids? In general, pesticides are probably most important when in interaction with other stressors, meaning they are an important factor in bee losses but not a major driver. This is speculative, of course, as the data are still too preliminary to draw strong conclusions.

Forage Availability

Several kinds of data suggest that lack of forage is a problem for honey bees (Vaudo et al., 2015). First, there are the reports from the beekeepers themselves that their colonies are starving (Steinhauer et al., 2014, vanEngelsdorp et al., 2011). Where our lab is in Davis, California, for example, we have a major problem with keeping bees alive due to starvation. The reason is rather simple: this dry landscape would naturally only support a small number of honey bee colonies. Our managed bees are kept in high numbers, however. This basic disconnect—that we are trying to keep organisms that are normally thinly dispersed in nature at high density—is sometimes missed in discussions of this topic. Second, it is easy to show that agricultural landscapes, in particular, are barren with respect to bee forage outside the time of flowering (Kremen et al., 2002, 2004, Klein et al., 2012, Dolezal et al., 2019b). This means farming areas are food deserts for bees for the overwhelming majority of the year. Third, large-scale land use patterns suggest a lack of forage even outside of farming areas due to land use choices and widespread use of herbicides (reviewed in vanEngelsdorp and Meixner, 2010). Finally, planting of monocultures may contribute to poor health given that bees can do poorly on less diverse pollen sources. Donkersley et al. (2014), for example, showed that protein content in bee bread correlates with land use patterns in the UK, with strongly agricultural landscapes being harsher environments for honey bees.

Many other studies show similar effects, and there is now considerable effort being made to improve the food landscape for pollinators via targeted planting of wildflowers (N. M. Williams et al., 2015, Lundin et al., 2017, Landis, 2017, Alaux et al., 2017, Garibaldi et al., 2017). Given the importance of flowering trees to honey bee foraging, it would be beneficial for these researchers to expand into advocating for the planting of these as well.

Interactions

The literature on the role of interactions between colony stressors (pesticides, parasites, pathogens, management practices) and colony losses is much like the literature on pesticides and colony losses. Most studies are laboratory based and use concentrations of pesticide, or other stressors, higher than what is found in the field (Collison et al., 2016). Field trials, or even large-scale studies in apiaries under controlled conditions in which multiple stressors have been tested against good controls, are lacking. Further, many of the results are contradictory, with one study showing an effect and another showing no effect for the same basic question (Siede et al., 2017, 2018). Given the context-dependent nature of the relevant effects, this is not a challenge to the positive results, or to the importance of the topic, so much as a call for more data to draw reasonable conclusions.

Having started with these caveats, the results from the lab, and the field, clearly suggest that interactions between stressors can be powerful and should contribute to losses (Dolezal et al., 2019a). In some sense, how could they not? The challenge is to quantify how big a problem particular forms of interaction are, and under what environmental conditions they occur. We review a representative set of studies to give the reader an idea of what has been done. The literature on this topic is rapidly growing, however, so it is likely that a review five years from now would be radically different. For now, the take-home message is that, until more field data come in, especially data with good controls, it is difficult to draw conclusions about the scope of the problem with respect to interactions and real-world colony losses.

Most work to date has focused on pesticides and Nosema. This is likely because dosages of both can be quantified. Vidau et al. (2011), for example, found that treatment of bees with thiacloprid increased Nosema spore counts relative to controls, while treatment with fipronil had the opposite effect. Pettis et al. (2013) found that levels of pesticides in pollen correlate with Nosema counts. With respect to pesticides and bacterial pathogens, Lopez et al. (2017)

recently showed that when bees are treated with pesticides prior to challenge with AFB spores, larvae show higher mortality than would be expected if the stressors were additive. Potentially explaining such results is the work by Brandt et al. (2016), which showed that neonicotinoids compromise the bee's immune system such that bees exposed to pesticides showed a weaker encapsulation response and decreased production of AMPs when challenged with pathogens. In a slightly more complex study, Doublet et al. (2015) showed an intricate set of interactions between pesticides, Nosema, and the virus BQCV.

Final Remarks on Large-Scale Colony Losses

Most recent reviews of this topic stress that a network of stressors impinge on bee health and are responsible for contemporary colony losses (Ellis et al., 2010, Genersch, 2010b, Becher et al., 2014, Henry et al., 2017). The accuracy of this viewpoint, however, is not clear. The outsized role of Varroa mites is only sometimes acknowledged (Le Conte et al., 2010, Neumann and Carreck, 2010, van Dooremalen et al., 2012, Smith et al., 2013). Mites cause direct damage, compromise the immune system, vector viruses, and must be controlled with toxic compounds put directly into the nest. They therefore cause what can appear to be a multifactorial effect associated with many stressors. In other words, it could be the case that *Varroa* alone is the major driver of colony losses. This is further supported by the fact that the current problem with large-scale colony losses started a few years after effective chemical control of *Varroa* began to fail (Thompson et al., 2002, Elzen, 2003, Elzen and Westervelt, 2004, Lodesani et al., 2005). This hypothesis about the role of mites is speculative, of course. It is stated strongly here to encourage more research to prove that this most parsimonious explanation is not correct.

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INDEX

absconding, 20, 295–296
adjuvants, 333–334
African bees, 15, 18, 157, 170, 184, 292, 296–297
Africanized bees: beekeeping and, 300, 344; biology of, 297–300; defensive behavior of, 291–293; genomics of, 170; interspecific competition and, 187; kin selection studies of, 166; nesting, 291; population biology of, 184; QTL studies in, 83–84; Varroa resistance of, 293–295
afterswarms, 139–141
aggression, Africanized versus European bees, 292; against beekeepers, 190–191; genetic basis of, 84, 114; in nestmate recognition, 269
alarm pheromone, 190, 242, 252, 266–267, 293
American foulbrood, 85, 148, 314–316
anarchistic bees, 158, 261
antenna, 48–50, 53, 63, 66, 94–96, 103, 115, 122, 220, 248, 252
antennal lobes: biogenic amines and, 216, 220; development of, 115; spatial processing in, 96–98; structure of, 91
antimicrobial peptides, 213, 302–305, 329, 351
Apis andreniformis. 11
Apis cerana, 12
Apis dorsata, 11–12
Apis florea, 11
Apis laboriosa. 11–12
Apis mellifera: domestication of, 18–22; evolutionary origin of, 16–18; genome of, 88–89; geographic range of, 12; GWAS studies in, 86; management of, 20–21; predators of, 190; subspecies of, 10–11, 13–16, 169–170; wild populations of, 18–19, 185
auditory processing, 95

bee bread, 66–68, 205. 209, 329–330, 349
beekeeping, artificial selection and, 19–20; commercial, 343–344; Darwinian, 312; domestication, 18–22; history of, 2; queenlessness and, 144; small hive beetle and, 314; supersedure and, 143; wax moths and, 314
Bombus, 171–172, 173, 180, 187, 324
brain: biogenic amines and, 216, 218–222; development, 37, 42, 150–151, 213; distributed physiology and, 202; endocrine system and, 62; gene expression in, 152, 173, 175; methylation of, 44; QMP and, 155; structure, 90–92
brood food, 34, 52, 177, 208, 213, 252, 264; nutritional content, 68–69
brood pheromone, 214, 223, 225, 242, 253–254, 255, 260, 262, 264, 279, 288
buzz run signal, 233, 246

caffeine, 327
capping cells, 39, 208, 262, 314
carbon dioxide, 248–249
caste determination: 36–46, epigenetics of, 37, 43–46; hormonal control of, 39–42, insulin signaling and, 42–43; role of microRNAs in, 37; role of sugars in, 36–37

chalkbrood, 148–149, 307–308, 316–317
circadian rhythm: behavioral studies of, 133; molecular genetics of, 110–112, 133–136; neurobiology of, 112–113
circulatory system, 61
cocoon, 34, 39–40, 192, 208
colony collapse disorder, 317, 319, 329, 338, 344–345
collective decision making, 277–278; nectar foraging, 272–275; nest choosing, 243–249; self-organization and, 239–240
comb, pattern formation on, 239–240
compound eyes, 49–50, 105
corpora allata, 62
corpora cardiaca, 62
crop, 55–56, 67, 71, 73–74, 92, 265, 273, 297

deformed wing virus, 308, 310–311, 313, 318, 320, 321
detoxification systems, 323–328
digestive system, 55–56
distributed Physiology, 202–203
division of labor: Africanized versus European bees, 291; biogenetic amines and, 216–220; genetic basis of, 220–225; physiological specialization associated with, 211–213; reproductive, 204–205; in workers, 205–210
drone congregation areas, 145, 256–257
drones: accessory glands of, 152–153; anatomy and physiology, 65–66; rearing of, 142–143; reproductive behavior of, 151–152

(E)-10-hydroxy-2-decenoic acid (10-HDA), 38, 44, 252, 257
eggs, 23–24
embryogenesis, 24–29
endocrine system, 39–40, 61–63, 91, 92, 113, 202
essential amino acids, 67
essential fatty acids, 68
European foulbrood, 316
eusociality: evolution of, 171–175; grades of, 9
evo-devo, 25–28
excretion, 323
exoskeleton, 60–61

fanning, 205, 264; temperature control, 236–237, 248–249; ventilation, 249
fat body, 56, 59, 150, 176, 193 ,220, 309; division of labor and, 213–215
flight muscles, 55, 92, 232, 247–248, 284, 339
foragers: assessment of patch quality, 276–277; improvement with experience, 284–286; nectar, 272–275; pollen, 276–282; role of octopamine, 217–218; water collection by, 282

genomic imprinting, 165–167
germ cell differentiation, 29
glands: Dufour's gland, 58–60, 64, 143, 151, 223, 257–258, 261–262, 270; hypopharyngeal, 36, 51–52, 60, 68–69, 72–73, 175, 180, 208, 257, 264, 270, 304, 329; Koschevnikov, 58, 60, 266; mandibular, 36, 51–52, 68, 141, 150–151, 154, 208, 213–214, 223, 256–258, 261, 265, 270, 304, 329; Nasonov, 59–60, 150, 252, 257, 264–265; salivary, 34, 52–53, 60, 91, 257, 270–271; venom, 58, 153, 176, 181, 257; wax, 59–60, 192–193, 209, 257
grooming invitation signal, 231–232
growth rate: Africanized versus European colonies, 290
guarding, 178, 191, 205, 209, 214, 242; robbing and, 188–189; specialization and, 235–236
gustation, 103–104

haplodiploidy, 23, 29, 87
head, 48
hemolymph, 56–57
heritability, 78
honey, 66–67

Hox genes, 25–28
hygienic behavior: Africanized bees and, 294; American foulbrood and, 316–317; caste and, 205, 209, 306; diseases resistance and, 307–308; genetic basis of, 84–86

imaginal discs, 34
immune system, 301–304
insulin signaling, 39–40, 42, 83, 170, 220–221
intercaste, 38–39
interspecific competition, 185–187
invertase, 67
Israeli acute paralysis virus, 304, 306, 319–321

Johnstone's organ, 94
juvenile hormone: caste differentiation and, 39–42; circadian rhythm and, 133; corpora allata and, 62; division of labor and, 177–178, 213–217, 222–223, 264; drones and, 152; laying workers and, 154; neurogenomics and, 113; queen reproduction and, 150–151; task allocation and, 139

kin recognition, 201
kin selection, 159–168
Kruppel homolog, 223–224

Langstroth hive, 19–20, 194
larvae, 33–34
laying workers, 65, 154–157, 164–165
learning: aversive, 219; caffeine and, 327; fatty acids and, 68; foraging and, 285; mushroom bodies and, 91, 97; olfactory, 98–102, 121–124; orientation and, 125, 128–130; pesticides and, 331–333; visual, 119–121, 232, 261
legs, 24, 53–54, 91–92, 103, 145, 193
life cycle, 9, 154, 182
lifespan: Africanized versus European, 290
local adaptation, 169–170

magnetic sense, 136
major royal jelly proteins, 69, 89, 180–181
Malpighian tubules, 56, 224
mating, 19, 66, 145–147, 150–151, 256–257
mating flights, 132, 143–144, 147, 149–152
mechanical senses, 93–95
Melipona, 8
memory: caffeine and, 327; mushroom bodies and, 91, 97; olfactory, 102–104, 114; pesticides and, 330–331, 333; visual, 121, 128
Mendelian traits, 76–77
mesosoma, 48, 53–55
messenger bees, 205, 208, 252, 258, 263
metasoma, 48, 55–60
microbiome, function of, 73–75; larval, 70; queen and drone, 72; worker, 70–72
middle-age bees, 200, 208, 217, 235, 240 248; caste and, 205–207; collective foraging and, 272–273; comb building and, 192–194; endocrine control of, 213–214; task repertoires of, 209–210
migratory behavior, 296–297
modulatory signaling, 233–235
molting, 33–34, 39, 42, 61–62
mouthparts: 50–52
mushroom bodies, 91, 97, 99–102, 107, 114, 150, 218

navigation, cognitive map, 130–132; image matching, 128–130
nectar, 67, digestive processing of, 67; toxic sources, 69–70
nectar guide, 119
neonicotinoids, 322, 325–328, 329, 331, 335–337, 349, 351
nest: Africanized versus European bees, 291; characteristics of, 191–192; finding a new, 243–246
nestmate recognition, 189, 218, 268–271
neurosecretory cells, 62
newly emerged bees, 70, 74–75, 208, 268, 286, 310, 318
nicotine, 326

Nosema, 304, 317, 319, 321, 350–351
nurses, 33, 34, 36, 52, 72, 112, 115, 151, 193, 212, 213, 240, 241, 279, 305, 316, 319; brood food and, 68–69; caste determination and, 38–39; circadian rhythm and, 133–135; division of labor and, 205–209; dopamine and, 218–220; juvenile hormone and, 214–216; microbiome and, 70–71; pheromones and, 223, 225, 264–265; reverted, 210–211; Varroa and, 309–310; vitellogenin and 177–178, 309–310
nutrition, 66–70, 349

ocelli, 50–51, 104
olfaction: discrimination and resolution, 123–124; learning, 98–101; neuroanatomy of, 95–97
ommatidia, 105–106, 117–118, 129
optic flow, 126–128
orchid bees, 8
orientation flights, 124–125, 132, 150, 151
ovaries, 41, 42, 44, 144, 158, 166, 176, 256; anatomy of, 64–65; laying workers and, 153–156, 164–165, 259–261; reproductive physiology and, 150–151

pars intercerebralis, 222
parthenogenesis, 16, 156–158
path integration, 125–128, 130, 132
patrolling, 194, 205, 207, 234, 240–242, 279
pattern formation: comb, 239–241
pesticides: in the comb, 329–330; interactions with other pesticides, interactions with other stressors, 350–351; 334–335; sublethal effects and, 330–333, 335–337
piping: in nesting finding, 245; queen, 233; worker, 232
polarized light, 105, 118, 126
pollen basket, 8, 38, 53, 63, 65
pollen hoarding bees, 80–84
pollination: basic biology of, 338–340; honey bees and, 341–344
polyandry: disease resistance and, 148; evolution of, 147–149; patriline consequences of, 77–78; task allocation and, 237–239; worker policing and, 160
population biology, 184–185, 290, 345–348
precocious foragers, 115, 133, 177, 210–211, 212, 213, 216, 220–221
prepupae, 7, 34, 40–41
proporoboscis extension reflex (PER), 83, 96, 122–124
propolis, 53, 73, 193, 194–195, 205, 209, 210, 303, 306, 315, 325
pupae, 34–35, 304, 306, 310, 319

QTL mapping, 78–86, 158, 292, 313
queen cups, 139, 143–144, 192, 263–264
queen duels: behaving during, 141–142; kin selection explanation for, 162–163
queen mandibular gland pheromone (QMP): anarchistic bees and, 158; glandular source of, 256–258; laying workers and, 154–155; as passenger pheromone, 251–252; and retinue response, 258–259; role in division of labor, 214–215, 218–220, 223, 225, 264; and sexual attraction, 257; suppression of workers ovaries, 260; and swarming, 262–264
queenless colonies, 24, 144, 160, 232, 258; reproduction in, 154–155, 260–261; selfish workers and, 164–165
queens: caste determination and, 36–46; egg laying, 23–24; growth as larvae, 33–34; mating, 19, 132; morphological differences relative to workers, 63–5; ovary suppression of workers by, 259–260; pheromones, 52, 60, 141; reproductive physiology, 66, 150–151; reproductive system, 64; sex determination and, 32–33; subspecies variation in, 13; virgin behavior of, 149–150

recombination, 87–88, 157
rectum, 56, 71
reverted nurses, 133, 210–211, 216
robbing, 187–189

royal jelly: microbiome and, 73; nutritional content of, 68–69; pheromones and, 252; role in caste determination, 36–46

scouts, 114, 139, 174, 188, 205, 233, 238, 243–246, 273–275, 277–278, 297
self-organization, 239–243
sex determination: molecular genetics of, 30–33; population genetics of, 168–169
shaking signal, 139, 205, 228, 231, 234, 275
small hive beetle, 314–315
social anatomy, 198
social detoxification, 329
social hormones, 254
social immunity, 306–307
social physiology, 198–199
solitary bees, 4, 6, 187, 202, 277, 320, 342
spermatheca, 64–65, 146–147
spermatogenesis: 29
stimulus response thresholds, 78, 235–237, 286–289
stinger, 38, 57–59, 63–64, 190
stingless bees, 5, 8, 10, 51, 147, 342
stop signal, 227, 228, 232, 246
subgenual organ, 53, 94
sun compass, 117, 126, 131–132
supersedure, 143–144, 261–262
swarming, 10, 149, 154, 182, 210, 228, 232, 233, 246, 261, 284, 295, 297, 312; kin selection and, 163; natural history of, 137–141; pheromonal control of, 262–263; queen duels and,142; rates, 184

tarsus, 53
taxonomically restricted genes, 175–176
thermoregulation: nest cooling, 248; winter warming, 247–248
tongue lashing, 205, 209, 248
trachea, 57, 61–62, 309
tracheal mites, 309
tremble dance, 200, 205, 228, 230, 231, 232, 234, 242, 275
trichoid sensilla, 93–94
trophallaxis, 50, 205, 206, 208, 279

ultraspiracle, 214–215
undertakers, 78, 178, 205, 214, 217, 235, 236, 306

Varroa mites: Africanized versus European bee resistance to, 293–295, 300: bee declines and, 345, 348, 351; biology of, 309–311; chemical ecology and, 271, 306; DWV and, 311, 318; hygienic behavior and, 307–308; pesticides and, 322, 325, 333; resistance of wild bees to, 311–314; RNAi control of, 320–321; robbing and, 187; wild bees and, 185, 311–314
venom: queens, 63–64; taxonomically restricted genes, 175; workers, 57–60
viruses, 317–320
visceral nervous system, 92
visual system: acuity of, 117–118; anatomy of, 104–106; color, 107–110, 116; and flowers, 118; polarized light, 126; processing, 106
vitellogenin, 155, 170, 172, 176–179, 181, 213, 214, 221

waggle dance: adaptive basis, 283–283; auditory processing, 95; biology of, 229–230; cognitive map and, 131; collective foraging and, 272–274; cues and, 200; genetic variation and, 237; magnetic sense and, 136; migration, 296; nest site selection and, 243–246; optic flow and, 127; pheromones and, 265; recruitment, 281; robbing and, 188; stop signal and, 227, 232; trichoid sensilla and, 6
wasps, 4, 32, 58, 129, 173, 180, 183, 199–200, 203, 339
wax, 50, 53, 59–60, 178, 205, 208–209, 235, 248, 257, 273, 306, 314, 315, 333; comb types, 191–192; pesticides and, 329–330; regulation of building, 192–194
wax moths, 314
wings, 53–55, 77, 92, 122, 229, 248, 264, 318
winter bees, 164
worker policing, 160–162